The Adverse Effect of Chemotherapy on the Developing Brain

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Overview of today’s talk

• Why study cancer survivors?

• Quick introduction on acute lymphoblastic leukemia

• Chemotherapy treatment

• The long-term effects of chemotherapy on the developing brain and associated functions

• Outstanding issues

• Clinical neuroimaging study

• Mouse neuroimaging

• What’s next?
WHY STUDY CANCER SURVIVORS?
Cancer survivors: a booming population

5-Year Relative Survival Rate for Acute Lymphoblastic Leukemia

Data obtained from Surveillance, Epidemiology, and End Result Program (SEER)

The burden of treatment

- Cancer treatment can pose a risk for long-term morbidities that affect many organs, including the brain

- Debilitating cognitive impairments that drive many survivors to seek additional care after being cured of cancer

- However, as this is a relatively new problem, survivors do not seem to receive appropriate care for their cognitive symptoms
  - For example, one study reports that stimulant medication may be ‘over-prescribed’ in ALL survivors given the incidence of attention/deficit hyperactivity disorder

- Why study survivors?
  - With improved treatment success and a growing population of survivors, medical professionals and pediatric ALL researchers are increasingly concerned with survivor quality of life
Acute lymphoblastic leukemia (ALL)

- ALL is a fast-growing type of blood cancer in which lymphoblasts (immature white blood cells) start to proliferate and replace normal blood cells of the bone marrow.
ALL is the most common type of childhood cancer
The incidence of ALL peaks in early childhood

Prognosis of survival is linked to age of diagnosis:

- Outcomes are poor for infant and adolescent ALL subtypes
- Outcomes are excellent for childhood ALL (diagnosed between 1 and 10)
A QUICK INTRODUCTION TO CHEMOTHERAPY TREATMENT
What does ‘chemotherapy’ involve?

- The success story in ALL cure rates is due to:
  - Pre-emptively treating the brain (to by-pass blood brain barrier)
  - Prolonging treatment for up ~3.5 years
    - (even when leukemic cells are no longer detected)
  - Include a number of chemotherapy agents:
    - Methotrexate (MTX)
    - Glucocorticoids
    - Vincristine
    - L-asparaginase
    - Anthracylnces
    - 6-merctopurine
    - Cytarabine
Impact of MTX on a cell

1. MTX enters the cell through the reduced folate carrier

2. Folate pools are depleted

3. Reduced DNA synthesis and production of cancer cells
How does cancer therapy relate to neuroscience?

• The basic principle of these types of therapies is that cancer cells divide at a faster rate than normal cell, they are hit harder by reductions in DNA synthesis

• However, there is plenty collateral damage to healthy cells

• This is where brain development enters our story
What makes childhood cancer patients particularly vulnerable to neurotoxicity?

Tremendous changes are taking place in the brain during early childhood.

Image credit: NIH MRI Study of Normal Brain Development
The changing brain

- Due to restrictions of the birth canal, babies’ brains are not fully developed when they are born

- Major changes in the brain take place AFTER birth

- ALL patients are exposed to chemotherapy treatment when dynamic changes in the brain are taking place

Image credit: NIH MRI Study of Normal Brain Development
Basic structure of the brain

Gray matter:
- cell bodies and dendrites

White matter:
- Axons and myelin

Cerebral spinal fluid (CSF):
- surrounds the brain and internal cavities
Brain contains lots of water molecules (protons).

Apply strong magnet: protons align in the direction of the magnetic field.

Radio frequency transmitter is turned on creating an electromagnetic field (EMF).

Protons in different tissue types have a different relaxation rate and this can be used to create images. E.g., Fat appears bright, fluids appear dark on a T1.

Frequency of EMF (resonance frequency) is absorbed and flip the spin of protons.

Receiver coils measure radio frequency generation during relaxation.

Magnetic Resonance Imaging.

Turn off EMF and protons spin back to normal (relaxation).
Neuroanatomical imaging

• Proton excitation depends on the type of oscillating magnetic field that is applied

• This feature is useful for looking at brain tissues

• T1-weighted images:
THE ADVERSE EFFECT OF CHEMOTHERAPY ON THE DEVELOPING BRAIN
Neurocognitive late effects in ALL survivors

- Late effects: Cognitive impairments after cancer treatment

- Impairment in executive functions (EFs) occur in 40 to 60% of ALL survivors
  - Working memory (remembering and manipulating information)
  - Attention problems (selective attention, sustained attention)
  - Processing speed (mental efficiency)
  - Inhibitory control (resistance on acting upon impulses)

- Why is this a big deal?
  - EFs are relevant for many aspects of life
    - EFs are impaired in many mental disorders (e.g., ADHD)
    - Poorer EFs are associated with poorer physical health (e.g., obesity, poor treatment adherence)
    - Poorer EFs are associated with reduced academic achievement and job success

- Poor EFs are associated with reduced quality of life
  - Relationship between cognitive impairments and quality of life in ALL survivors
  - Keep in mind – these individuals will have to live with these impairments for a lifetime
Chemotherapy is toxic to the brain

Quantitative neuroimaging studies also reveal reduced white matter volume and altered white matter structure as measured with diffusion tensor imaging (DTI), compared to healthy comparisons.

These two 14-year old ALL survivors were at least 3 years off treatment when they went into the scanner.
Possible mechanisms how MTX may affect the brain

1. MTX blocks folate

2. Methionine-homocysteine pathway goes unchecked

3. Homocysteine builds up to toxic proportions

4. Choline may be recruited away from the membrane as compensation, leaving phospholipids vulnerable
Possible mechanisms how MTX may affect the brain

- Excess of homocysteine is toxic to cell
  - Vascular damage

- Choline is essential for membrane phospholipids that are found in gray matter and white matter
  - Demand for choline high during early childhood years of development
Outstanding issues

• How do we improve quality of life in cancer survivors?
  • One approach to address this is figuring out how do cancer therapies lead to abnormalities in the brain and impairments in cognitive functions
  • Still not so easy to answer…
    • Neurocognitive late effects arise from a confluence of oncology, genetics, psychology and dynamic changes in the brain,
• Translational research
  • Basic research can illuminate the biological and physical principles of how chemotherapy exerts its effects on the brain
  • Translational or applied research is essential for shedding light on how these biological and physical principles apply to people
    • You cannot ask mice how things are going

• Integrative, multidisciplinary is essential for understanding how neurocognitive late effects come about, which in turn can tell us how way may be able to prevent late effects from happening
PRELIMINARY FINDINGS OF SELECTION OF STUDIES WE PREFORMED SO FAR
Clinical study: Which brain regions are affected by chemotherapy and how do changes relate to cognitive impairments?

- Establish neurocognitive late effects in cancer survivors
- Establish brain changes after chemotherapy exposure
- Establish how brain changes relate to cognitive function and quality of life
NPhenoGENICS: Neurocognitive-Phenome, Genome, Epigenome and Nutriome In Childhood Leukemia Survivors

• Purpose: To find possible therapeutic targets to help prevent neurocognitive side effects of chemotherapy in survivors of childhood leukemia

• Neurocognitive-phenome:
  • Wechsler Intelligence Scale
    • Visual-motor speed: Coding
    • Manipulation of verbal information in temporal storage: Digit Span Backward
    • Short-term auditory memory: Digit Span Forward
    • Non-verbal processing speed: Symbol Search
  • Math performance
  • ADHD
Normed tests

Compare the sample's mean to the population mean.

Compare the proportion of the sample that is clinically impaired to what we would expect from the general population.
Study 1: How do ALL survivors compare to the general population?

**Total sample: 76**

<table>
<thead>
<tr>
<th>Number of Males:</th>
<th>Number of Females:</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 46</td>
<td>• 30</td>
<td>• 8.66 – 18.87</td>
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<tr>
<td></td>
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<td>• Mean: 13.63</td>
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Age at Diagnosis of the Sample

Majority was diagnosed between 2 and 5 years of age

Frequency

Age At Diagnosis

0 2 4 6 8 10 12

How do ALL survivors compare to the normal population in terms of cognitive functioning?

NEUROCOGNITIVE-PHENOME: PRELIMINARY RESULTS
Study 1: Wechsler Intelligence Scale Results – Subtests

On average, ALL survivors score significantly lower than the population mean for Coding (processing speed) and Digit Span Backward (working memory)
Study 1: Wechsler Intelligence Scale Results - Composite Scores

- Impairments in ALL survivors seem clustered in speed of processing and working memory, while verbal comprehension and perceptual reasoning are intact.
Variable outcomes

Majority of survivors are in the lower range, but some have excellent scores.
What about the survivors who received high-intensity treatment?
Math Performance

- While there is a trend towards impaired performance, the difference is borderline significant only.
- There is huge variation amongst survivors!
Means versus Risk

- **ALL survivors have a higher risk than those in the general population to have math problems that meet clinical criteria.**
ALL survivors are more likely to have ADHD compared to the general population

- The risk of developing ADHD is significantly increased in ALL survivors than what we would expect from norm \( (t = 3.47, p < 0.001) \)
- Treatment intensity does not affect ADHD outcomes \( (p > .6) \)

22% of ALL survivors are within the clinical range (shaded areas)
What do these results tell us?

• How do ALL survivors compare to the normal population in terms of cognitive functioning?

• ALL survivors exhibit slower processing speed and impaired working memory

• ALL survivors have a substantially higher risk than individuals in the general population to be clinically impaired
  • Math
  • ADHD

• Variability!
Variability…

- Some survivors do not seem to experience long-term side effects from chemotherapy treatment, while others do
  - Treatment intensity does not necessarily explain variability

- Genetic predisposition?
  - Presence of less-efficient’ alleles associated with poorer cognitive performance in MTX-exposed ALL survivors

This DNA profile may leave some individuals more vulnerable than others.
What kind of effect does exposure to chemotherapy have on the developing brain?

NEUROIMAGING
Sample Characteristics

- Sub-sample of the NPhenoGENICS study (boys only)

- This study includes controls
  - 18 ALL survivors and 13 Controls
  - Age range: 8.66 – 17.86
  - Mean age: 14.41
Clinical Neuroimaging Study

Pre-processing: Reducing error/noise
Registration: Align images such that common features overlap

Post-Processing
Masking of non-brain voxels
Tissue classification

*The results are on the T1 weighted images
Incidental findings

- Incidental finding rate of 33% in the ALL survivor group
Preliminary Imaging Results: Regional Gray Matter Significantly Reduced in Survivors

*White matter significantly reduced in the frontal lobes only
Correlating Brain Structure with Cognitive Functions

• Survivors in this sample also show circumscribed cognitive deficits
  • Response cancellation or the ability to inhibit an ongoing response
  • We will correlate these ‘cognitive phenotypes’ with brain structure
Clinical Neuroimaging Summary

- What kind of effect does chemotherapy exposure have on the developing brain?
  - Exposure to chemotherapy alters brain structure
  - Literature has emphasized white matter vulnerability, but we see gray matter
  - We predict that cognitive deficits correlate with abnormalities in brain structure
How does methotrexate chemotherapy affect the developing brain?

MOUSE NEUROIMAGING
Why Mouse Models of Cancer Care?

- The obvious: Trying out how each chemotherapy agent works in isolation is unethical to test in children

- The benefits of mouse models:
  - Systematic experimentation:
    - Keep all variables constant, expect for variable of interest (e.g., chemotherapy agent)
  - Efficiency:
    - Mice develop fast: Characterize a life-time worth of brain changes in several weeks
    - Imaging cohorts at the time
  - Genetically-modified mouse strains:
    - Explore how genetic mutations affect outcomes
  - Using similar imaging techniques as in the human study allows us to connect the neurodevelopmental changes observed in survivors to individual chemotherapy agents
Mouse Neuroimaging

A: Axial Plane
B: Sagittal Plane
C: Coronal Plane

- Image contrast is enhanced by injecting mice with manganese
Image Analysis

- All images for each subject are forced into a “consensus average”
Experimental Design

- 13 Mice were treated with low dose MTX (20 mg/kg)
- 9 Mice were the saline controls
MTX results in changes in brain development, even at a low dose

- Volumetric reductions were significant for the anterior commissure (plot), occipital cortex, mamillary bodies, pontine nucleus, subiculum and cerebellar peduncle
- Hippocampus borderline significant
Mouse Imaging Summary

- MTX is delivered in large amounts, over a long period of time, and via various routes in ALL patients

- Even a one-time injection of MTX at a low dose resulted in volumetric deficits across various regions at P42 (adolescence)

- Give the gradual nature of the deficits, we may be able to intervene and prevent impairments
What’s next?

• Genetic risk
  • Identification of genetic variants that pose a risk for neurocognitive late effects
  • Treating genetically-altered mice with chemotherapy agents
    • E.g., folate deficient mice

• Rescue treatment
  • A mouse study in which we try to reverse the adverse impact of MTX by way of choline supplementation
THANK YOU!!!

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