“FROM NATURE TO THE CLINIC-MANAGING SIDE EFFECTS OF RADIOTHERAPY FOR CANCER TREATMENT”

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1. Introduce oxidative lung damage.

2. Radiation toxicity of normal lung tissues

3. Natural products and their use in mitigating radiation lung damage.

4. Modeling lung toxicity in rodents to study adverse effects of therapeutic radiation

5. Designing Effective Radioprotecting agents
An immediate effect of tissue irradiation is the generation of reactive oxygen (ROS) and nitrogen (RNS) species that can produce oxidative damage to DNA, lipids, and proteins resulting in cell injury or death.

The lung is one of the most sensitive tissues to ionizing radiation, and damage to normal lung tissue remains a major obstacle in the treatment of a variety of cancers.
Oxidative/Nitrosative Stress and Tissue Damage

Enzymatic and nonenzymatic antioxidants (endogenous tissue defense) detoxify ROS and RNS and minimize damage to biomolecules.

An imbalance between the production of ROS/RNS and antioxidant capacity leads to "oxidative stress" that contributes to the pathogenesis of radiation-induced tissue damage by damaging lipids, protein, and DNA.
Radiation Pneumonopathy

Radiation Therapy is commonly used to treat lung cancer and other thoracic malignancies (mesothelioma, breast cancer, esophageal cancer, lymphomas).

Up to 30% of patients irradiated for lung cancer and 10-15% of other thoracic oncology patients develop clinically significant radiation lung injury.

Radiation Damage to the Lung is characterized by:
A) Pneumonia-like symptoms (inflammation)
B) Fibrotic lung damage (irreversible).
### Scoring of Clinical Manifestations of Radiation Damage of Lung Tissues

**RTOG/EORTC Late Radiation Morbidity Scoring Schema**

<table>
<thead>
<tr>
<th>ORGAN TISSUE</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Grade 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>LUNG</td>
<td>None</td>
<td>Asymptomatic or mild symptoms (dry cough) Slight radiographic appearances</td>
<td>Moderate symptomatic fibrosis or pneumonitis (severe cough) Low grade fever Patchy radiographic appearances</td>
<td>Severe symptomatic fibrosis or pneumonitis Dense radiographic changes</td>
<td>Severe respiratory insufficiency / Continuous O₂/ Assisted ventilation</td>
</tr>
</tbody>
</table>

Current Readings: Improvements in Intensity-Modulated Radiation Therapy for Malignant Pleural Mesothelioma

Table 1. Comparison of Dosimetric Parameters, Toxicity, and Outcome in Patients Receiving IMRT After Extrapleural Pneumonectomy

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of Patients</th>
<th>Technique</th>
<th>Dose (Gy)</th>
<th>Boost (Gy)</th>
<th>V5 (%)</th>
<th>V20 (%)</th>
<th>Mean Lung Dose (Gy)</th>
<th>Grade 3 + RP (%)</th>
<th>Grade 5 RP (%)</th>
<th>2-Year OS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giraud et al(^9)</td>
<td>24</td>
<td>HT</td>
<td>50</td>
<td>4-6</td>
<td>99</td>
<td>4</td>
<td>11</td>
<td>16</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>Patel et al(^8)</td>
<td>30</td>
<td>IMRT</td>
<td>45</td>
<td>8-25</td>
<td>56</td>
<td>4</td>
<td>7.2</td>
<td>13</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Gomez et al(^7)</td>
<td>86</td>
<td>IMRT</td>
<td>45-50</td>
<td>10</td>
<td>8</td>
<td></td>
<td></td>
<td>12</td>
<td>6</td>
<td>32</td>
</tr>
</tbody>
</table>

Gy, Gray; V5 and V20, dose of lung receiving 5 and 20 Gy, respectively; RP, radiation pneumonitis; OS, overall survival.
Incidence of Radiation Pneumonitis (RP) in Relation to V20 and Effects of Concurrent Chemoradiation.

Patients receiving chemotherapy had a sharper increase in risk of radiation pneumonitis as the volume of normal lung exposed to 20 Gy increased.
Radiation Toxicity to Normal Tissues

The usefulness of thoracic radiotherapy in the treatment of cancer is greatly limited by toxicity of ionizing radiation (radiation pneumonopathy).

Therefore, if we protect “normal” lung parenchyma from radiation injury, we will increase the ability to deliver tumoricidal radiotherapy doses.
Despite active research in the development of tissue radioprotectors, there is no known effective pharmacologic therapy for the prevention of radiation pneumonopathy.

Steroids are used to treat Acute radiation pneumonitis, but do not alter risk of developing long term, Chronic, fibrotic complications.
An expanding body of preclinical evidence suggests that a number of botanicals have the potential to impact a variety of human diseases including lung disease.

Therefore, non-toxic natural agents could be useful either alone or in combination with conventional therapeutics for the prevention or therapy of oxidative lung disease.
Annual sale of Medicinal Herbs in the US is > 3 Billion $$$

More than 60 million consumers in the U.S. take herbal remedies. More physicians are recommending herbal medicines and some health insurance plans offer coverage for alternative health treatments such as herbal remedies.

In 1993 the NIH opened the National Center for Complementary and Alternative Medicine (NCCAM), now NCCIH, which along with the Office of Dietary Supplements (ODS) aim to promote the safety, effectiveness, and biological action of botanical products.
Complementary & Integrative Health Approaches

**Natural Products**
This group includes a variety of products, such as herbs (also known as botanicals), vitamins and minerals, and probiotics. They are widely marketed, readily available to consumers, and often sold as **dietary supplements**.

**Mind and Body Practices**
Mind and body practices include a large and diverse group of procedures or techniques administered or taught by a trained practitioner or teacher.

**Other Complementary Health Approaches**
The practices of traditional healers, Ayurvedic medicine from India, traditional Chinese medicine, homeopathy, and naturopathy.
The Ten Most Common Complementary Health Approaches Among Adults (2007)

Barnes et al, 2007; CDC National Health Statistics Report#12, 2008

NATURAL PRODUCTS
Gullet et al., 2010
Molecular Pathways Affected by Common Botanicals

Gullet et. al, 2010
Drug Development From Bioactive Dietary Agents

Dietary Antioxidants (Vegetables and Fruits) → Purification of Main Active Components → Evaluation in Cell Culture and Animal Models → Elucidation of Mechanism of Action

DRUG DEVELOPMENT → Testing in Clinical Trials → Determining Bioavailability/Biospecificity
Botanicals with antioxidant properties currently being evaluated in lung disease and cancer

Dietary Agents

Green Tea

Turmeric

Flaxseed

Chemical Structures

Epigallocatechin-3-gallate (EGCG)

Curcumin

Secoisolariciresinol Diglucoside (SDG)

Grapes

Tomatoes

Pomegranate

Broccoli

Dietary Agents

Chemical Structures

Resveratrol

Lycopene

Delphinidin

Sulforaphane
Flaxseed: “an ancient remedy in a modern world”
The father of modern medicine, Hippocrates, the Greek physician, by 650 B.C. wrote about the use of flax to relieve inflammation of mucous membrane and for the relief of abdominal pains and diarrhea.

By the 8th Century A.D. Charlemagne, one of the greatest medieval kings, considered flax so important that for the health of his subjects he passed laws and regulations requiring its consumption.

Mahatma Ghandi said that when flaxseed was added to people's diet, their health improved.
Flaxseed - a Natural Product

Flaxseed flower → Flaxseed Seed-Capsules → Flaxseed Seeds → Finely Ground Flaxseed

Flaxseed Oil Omega-3 FA

Lignan SDG
FLAXSEED

Plant Lignan Precursors
- Secoisolarisiresinol diglycoside (SDG)
- matairesinol

Intestinal Bacteria

Lignans
- Enterodiol (ED)
- Enterolactone (EL)

Biological Properties
*** Antioxidative
Antiproliferative
Antiangiogenic
Estrogenic/Antiestrogenic

Cancer Protection

Omega-3 Fatty Acids
- \( \alpha \)-Linolenic Acid
- EPA (eicosapentanoic Acid)
- DHA (Docosahexanoic Acid)

** Anti-inflammatory
Flaxseed Lignan Structure

- Flaxseed Lignan Structure
- Resveratrol
- Curcumin
- Quercetin
- Secoisolariciresinol diglucoside (SDG)

A bi-phenolic with potent antioxidant properties
Our Group Has Identified Flaxseed As A Potent Inhibitor Of Oxidative Lung Injury In A Number Of Animal Models
Protective Properties of Flaxseed in Preclinical Models of Cancer & Acute/Chronic Lung Damage

- FLAXSEED (wholegrain) & SDG
- THORACIC RADIATION PNEUMONOPATHY
- HYPOXIC LUNG DAMAGE
- ISCHEMIA-REPERFUSION LUNG DAMAGE
- ACID ASPIRATION-INDUCED LUNG DAMAGE
- ASBESTOS-INDUCED MESOTHELIOMA
- TOBACCO CARCINOGEN-INDUCED LUNG CANCER
Direct Free Radical Scavenging by Flaxseed Lignan-Antioxidant action in γ-irradiated lung Endothelial cells

Lee et.al, 2009
Genetic profiling of flaxseed in lung
(30,000 gene array of entire mouse genome)

6.8% of all mouse genes in lung tissues are significantly modified by flaxseed

Dukes et al., 2012
### Pulmonary Gene Expression Profiling of Genes With >1.5x fold Change in Individual Flax-fed Mice as Compared to Mean of Control

Red indicates up-regulation, green down-regulation

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Dukes et.al, 2012
Given the direct free radical scavenging properties of the flaxseed lignans and the robust boost of antioxidant tissue defenses, we hypothesized that dietary flaxseed and will ameliorate oxidative acute and chronic lung damage such as that resulting from radiation exposure, modeled in mice.
Mouse Model of Thoracic Radiation Damage
Mouse Radiographs

Cephalic margin

Caudal margin

(XRT=X-Ray Treatment)
Dietary Flaxseed Ameliorates Radiation-Induced Pneumonitis (Inflammation) in Mice

Lee et al., 2009
Antifibrotic Role of Flaxseed

Flaxseed Decreased Radiation-Induced Collagen Deposition in Lungs

Fibrotic Index (Pathology)

OH-Proline Content

Trichrome Blue Staining for Collagen (Marker for Lung Fibrosis)

Cancer Biology and Therapy, 2009
Flaxseed Does Not Impair Tumor Eradication By Radiation

Christofidou-Solomidou et al., Radiation Research, 2012
Summary

Dietary Flaxseed:
• Improves Survival
• Prevents Radiation-induced
  • Oxidative Tissue Injury
  • Pneumonitis
  • Inflammation
  • Lung Fibrosis
  • Cytokine Secretion
• Does NOT protect Tumor
FLAXSEED SUPPLEMENTATION

- Breast Cancer
- Colon Cancer
- Ovarian Cancer
- Prostate Cancer
- Cystic Fibrosis (UPENN)
- Radiation Damage (UPENN)
- Lung Transplantation (UPENN)
- Cigarette Smokers (UPENN)
- Diabetes
- High Cholesterol
- Hypertension
DIETARY FLAXSEED IS WELL TOLERATED BY HEALTHY VOLUNTEERS AND CYSTIC FIBROSIS PATIENTS

Dietary Flaxseed (40g daily) Supplementation

Plasma Lignan Concentration Increases after Flaxseed Consumption

Future Plans: Determine if Flaxseed Supplementation Modulates systemic inflammation and disease exacerbations in CF patients

Turowski et al, in review
The working paradigm of mesothelioma carcinogenesis is that asbestos induces a state of chronic inflammation in the pleura that ultimately leads to mutagenesis and tumor formation (especially in those with a genetic predisposition).

**Key roles of:**
HMGB1, TNFα, IL-1β
AND
REACTIVE OXYGEN SPECIES
Hypothesis

Inhibition of inflammation and/or ROS will delay or prevent the induction of asbestos-induced mesothelioma.

We want to test this using Flaxseed and the main lignan found in Flaxseed: the SDG.
University of Pennsylvania receives $10M to study Superfund asbestos site

July 11, 2014 9:52 AM
By HEATHER ISRINGHAUSEN GVILLO

PHILADELPHIA (Legal Newsline) – Researchers with the University of Pennsylvania have received a $10 million grant to study asbestos and how the toxic fiber leads to mesothelioma and six other asbestos-related diseases at America’s 10 Superfund sites.

The grant, which came from the National Institute of Environmental Health Sciences, will help researchers from the school’s Center of Excellence in Environmental Toxicology in the Perelman School of Medicine to study asbestos, mesothelioma, and other asbestos-related diseases over the next four years.

University of Pennsylvania researchers receive $10 million to study asbestos in Ambler

Published: Tuesday, June 24, 2014
By Eric Devlin
edevlin@montgomerynews.com

The University of Pennsylvania recently announced it has received a $10 million grant from the National Institute of Environmental Health Sciences to study asbestos and its impact on the Ambler community.

The grant will allow researchers from Penn’s Center of Excellence in Environmental Toxicology to, over the next four years, study asbestos, the rare asbestos-related cancer, mesothelioma, and other asbestos-related diseases, according to a press release. Researchers from the Abramson Cancer Center, the Penn School of Arts and Sciences and Fox Chase Cancer Center are also lead investigators on the grant.

The BoRit site where research will take place, located in Ambler Borough, Upper Dublin and Whitemarsh townships between Butler Avenue, North Maple Street and the Wissahickon Creek, was placed on the Environmental Protection Agency’s Superfund National Priorities List in April 2009.
1. Can we remediate asbestos without moving it from the original disposal site?

2. What do we know about the fate and transport of asbestos in the environment by water and air?

3. What do we know about the exposure pathways that were responsible for the mesothelioma cluster in Ambler? And why is the incidence higher in women?

4. Is susceptibility to mesothelioma genetic?

5. Can asbestos-related disease be prevented?

6. Is there a blood test to determine whether a person will get asbestos-related disease?
USEFULNESS OF FLAXSEED TO PREVENT MM FROM ASBESTOS EXPOSURE
Role of Flaxseed and SDG in Preventing Asbestos-Induced Mesothelioma in Mice

We hypothesize that SDG or flaxseed diets will decreased asbestos induced ROS/inflammation leading to: 1) ROS, 2) decreased cytokines, 3) decreased HMGB1, 4) less tumorigenic foci, and 5) less tumors.
EXPOSING CELLS TO ASBESTOS

1. ROS levels using H₂DCFDA
2. Supernatant → Cytokine (TNF-α; IL-1β)
3. Cells → Inflammasome activation
4. MDA (Lipid Peroxidation)
5. Nitrite/Nitrate levels
SDG given to Macrophages Post Asbestos-Exposure Decreases Oxidative Stress and Inflammatory Cytokines

**Oxidative Stress**

- Media MDA Concentration (µM)

**IL-1β**

- Media IL-1β Concentration (pg/ml)
Summary of Findings

1. SDG blocks asbestos-induced ROS macrophages.
2. SDG blocks inflammatory cytokine secretion by mouse peritoneal macrophages exposed to asbestos.
3. SDG blocks oxidative (lipid peroxidation) and nitrosative stress (nitrite levels) in mouse peritoneal macrophages exposed to asbestos.

Findings from cell experiments justify pre-clinical experimentation to determine the usefulness of flaxseed and its lignan SDG in blunting chronic inflammation and ultimately malignancy due to asbestos exposure.
Using 2 models of mice genetically predisposed to develop mesothelioma after asbestos exposure, we will: Evaluate the acute effects of Flaxseed and SDG on asbestos exposed mice; test whether Flaxseed and SDG inhibits the development of tumors in genetic models of accelerated, asbestos induced MM.
Data from this work will provide important evidence for the usefulness of this bioactive natural product in blunting cancer development from asbestos exposure and provide insight in the mechanisms involved.

If our studies show efficacy with safety, our long-term goal would be the evaluation of Flaxseed and SDG as chemopreventive agents for mesothelioma in exposed populations.
Funding Provided by:

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RC1AI081251

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