“The synthetic lignan Secoisolaricirecinol Diglucoside (LGM2605) prevents asbestos-induced inflammasome activation and cytokine secretion in murine macrophages”

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Professor of Medicine
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DISCLOSURES

- Dr. Christofidou-Solomidou reports grants from:
  - National Cancer Institute (NCI),
  - National Institute of Environmental Health Sciences (NIEHS),
  - National Institute of Allergy and Infectious Diseases (NIAID)
  - National Institute for Mental Health (NIMH) and
  - National Center for Complementary and Integrative Health (NCCIH).
  - National Aeronautics and Space Administration (NASA)

- Dr. Christofidou-Solomidou has a patent No. PCT/US14/41636 pending, and patent No.PCT/US15/22501 pending.

- Dr. Christofidou-Solomidou is founder of LignaMed LLC, devoted to developing radioprotective agents.
Our Research Team
The BoRit Asbestos Site was used, from the early 1900s to the late 1960s, to dispose of asbestos-containing material (ACM) that came from a nearby asbestos products manufacturing plant.
The Ambler zip code had 28 cases of mesothelioma rather than the expected 9 for a population of its size - about 30,000.

The BoRit site was added to the EPA's National Priorities List of the most hazardous waste sites on April 9, 2009, making it eligible for cleanup using federal Superfund program funding.

University of Pennsylvania receives $10M to study Superfund asbestos site

PHILADELPHIA (Legal Newsline) – Researchers with the University of Pennsylvania received a $10 million grant to study asbestos and how the toxic fiber leads to asbestos-related disease in response to America’s 10 Superfund sites.

The grant, which came from the National Institute of Environmental Health Sciences (NIEHS), is expected to help researchers from the school’s Center of Excellence in Environmental Toxicology (CEET) at the Perelman School of Medicine to study asbestos, mesothelioma and other asbestos-related diseases over the next four years.
Asbestos Exposure and Malignant Mesothelioma

- Asbestos fiber inhalation can lead to malignant mesothelioma, lung cancer, as well as pulmonary fibrosis.
- MM is a highly aggressive cancer that arises from the mesothelial cells of the pleura and peritoneum with a median survival of about 1 year.

- Current therapies, other than surgery in very early disease, are not curative.

Presently, MM causes about 3,000 deaths per year in the US and an additional 5,000 deaths/year in Western Europe.
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, TNF-α, IL-1β, IL-18, TGF-β1 AND REACTIVE OXYGEN SPECIES
From the cellular perspective, asbestos induces chronic production of reactive oxygen species (ROS) which results in chronic pulmonary inflammation and cytokine (i.e. TNF$\alpha$, HMGB1, IL-1$\beta$) release through ROS-mediated activation of NF$\kappa$B and through inflammasome activation.
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
Protective Properties of Flaxseed & SDG in Preclinical Models of Cancer & Acute/Chronic Lung Damage

FLAXSEED (wholegrain) & SDG

THORACIC RADIATION PNEUMONOPATHY
HYPEROXIC LUNG DAMAGE
ISCHEMIA-REPERFUSION LUNG DAMAGE
ACID ASPIRATION-INDUCED LUNG DAMAGE
TOBACCO CARCINOGEN-INDUCED LUNG CANCER
ASBESTOS-DISEASES
Manufactured in scalable amounts that now allow *in vivo* evaluation
Flaxseed Lignan SDG blunts B[a]P-Induced ROS

Testing SDG in ROS scavenging in lung epithelial cells exposed to the tobacco carcinogen benzo-alpha-pyrene (B[a]P)
We hypothesize that flaxseed or SDG-rich diets will decrease asbestos induced ROS/inflammation leading to: 1) ROS, 2) decreased cytokines, 3) decreased HMGB1, 4) less tumorigenic foci, and 5) less tumors.
Experimental Plan

1. ROS levels using H$_2$DCFDA
2. Supernatant $\rightarrow$ Cytokine (TNF-$\alpha$; IL-1$\beta$)
3. Cells $\rightarrow$ Inflammasome activation
4. MDA (Lipid Peroxidation)
5. Nitrite/Nitrate levels
SDG scavenges ROS and prevents asbestos-induced cytotoxicity & oxidative cell damage

**ROS**

- 20µg Asbestos per cm²
- Time (Hours) Post-Asbestos
- Arbitrary Units of Fluorescence

**H₂O₂**

- Cell Culture Media
- Time (Hour) Post-Asbestos
- H₂O₂ Concentration (µM)

**LDH**

- CTL
- SDG
- ASB + SDG
- LDH Cytotoxicity (A₅₉₀ - 650 nm)
- Time (Hour) Post-Asbestos

**Oxo-G**

- CTL
- SDG
- ASB
- ASB + SDG
- Cell Culture Media Oxidized Guanine Species (pg/ml)
- Time (Hour) Post-Asbestos

*Pietrofesa et al, IJMS, 2016*
SDG mitigates asbestos-induced inflammation and oxidative stress

**SDG given post-asbestos**

- **IL-1β**
- **TNFα**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IL-1β Concentration (pg/ml)</th>
<th>TNFα Concentration (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL</td>
<td>1000</td>
<td>50</td>
</tr>
<tr>
<td>SDG(25)</td>
<td>500</td>
<td>70</td>
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<tr>
<td>SDG(50)</td>
<td>700</td>
<td>90</td>
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<td>Asb x3</td>
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<td>130</td>
</tr>
<tr>
<td>Asb x8</td>
<td>1900</td>
<td>140</td>
</tr>
</tbody>
</table>

* indicates significant difference from control
# indicates significant difference from SDG(25)
Asbestos Exposure Induces the Expression of Phase II Antioxidant Enzymes

Elicited murine peritoneal macrophages were harvested after 0, 0.5, 1, 2, 4, 5, 8, 12, and 24 hours of asbestos exposure and evaluated by western blotting for (a and b) HO-1 and (a and c) Nqo1.

Pietrofesa et al, IJMS, 2016
SDG (LGM2605) Boosts Asbestos-induced Expression of Nrf2-regulated Antioxidant Enzymes

(a) Levels of active, nuclear Nrf2 were determined at 0, 1, 2, 4, 6, 8, and 12-hours post asbestos exposure.

(b) Macrophage mRNA expression of HO-1.

(c) mRNA expression levels of NQO1 determined at 0, 8, and 24 hours post asbestos exposure using qPCR.

Levels of antioxidant enzymes were determined by (a) western blotting for HO-1 and Nqo1. Densitometric analysis of band intensity for (b) HO-1 and (c) Nqo1 was normalized to β-actin and values are expressed as fold change from CTL at time 0.

Pietrofesa et al, IJMS, 2016
Publication of Findings

Article
Asbestos Induces Oxidative Stress and Activation of Nrf2 Signaling in Murine Macrophages: Chemopreventive Role of the Synthetic Lignan Secoisolariciresinol Diglucoside (LGM2605)

Ralph A. Pietrofesa, Anastasia Velalopoulou, Steven M. Albelda and Melpo Christofidou-Solomidou *
Inflammasomes are specialized inflammatory signaling platforms that govern the maturation and secretion of proinflammatory cytokines, such as IL-1β and IL-18, through the regulation of caspase-1-dependent proteolytic processing.

Inhalation of fibers like asbestos can induce the formation of the NLRP3 inflammasome.
The NLRP3 inflammasome responds to activating signals through a two-step activation model. Initiation is typically triggered by ligand binding (e.g., LPS) to Toll-like receptors (TLR; e.g., TLR4) and related receptors. This results in activation of NF-κB, which translocates to the nucleus and activates the transcription of inflammasome components, including NLRP3, and the pro forms of inflammasome-related cytokines (i.e., pro–IL-1β and pro–IL-18).

1. Activation of inflammasome-associated caspase-1 results in the activation (cleavage) of proinflammatory cytokines (e.g., IL-1β, IL-18) before their secretion.
Pietrofesa et al, in review
SDG (LGM2605) prevents NF-κB Activation in Peritoneal Macrophages - (1)

Pietrofesa et al, in review
SDG (LGM2605) prevents Asbestos-Induced Inflammasome Activation in Macrophages-(2)
SDG (LGM2605) prevents Asbestos-Induced Inflammasome Activation in Macrophages

**Graphs:**
- **NLRP3 Fluorescence Levels:**
  - 8 Hours Post-ASB.

- **NLRP3 Fold Change from CTL:**

**Image:**
- Western blot analysis of NLRP3 and β-Actin showing bands at 110 KDa and 42 KDa, respectively.

*Pietrofesa et al, in review*
SDG (LGM2605) prevents Inflammasome-regulated Cytokine Secretion in Macrophages-(3)

Pietrofesa et al, in review
Proposed Mechanisms of SDG (LGM2605) Protection from Asbestos-Induced Cell Damage and Death

Pietrofesa et al, in review
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