DISCOVERY AND CLINICAL EFFECTIVENESS OF A COMPOSITION THAT PROMOTES HAIR GROWTH (PATENT PENDING)

Hiroshi Okawa
Pharmacy researcher of Hokkaido Pharmaceutical University School of Pharmacy
Representative Director of Scarecrow Inc., Tokyo, Japan
1. Development Background
2. Clinical Trial Data
3. Putative Mechanisms of Action
1. Development Background

- Development Background

- Rationale for Selecting Ingredients:
  “Lipopolysaccharide (LPS)” and “pine bark polyphenol”
Development  Background

- Modern Pet disease
  - Heart disease
  - Cancer
  - atopic dermatitis and alopecia etc

- The reason of appearance
  - Stress
  - High calorie of pet food and lack of physical exercises
  - Pet clothes
  - Aging etc
Rationale for Selecting Ingredients

- Limit of pharmaceuticals
- Side effects of medicine
- Start caring before occurring a disease

To focus on the action “Immune function”
Hygiene hypothesis

Many brothers and sisters
Country life with animals

Only one child
Urban life

Most significantly different factor is the exposure amount of endotoxin (LPS) in life.

Hygiene hypothesis: a study of canine atopic dermatitis

This study suggests that there is a protective effect of high indoor endotoxin exposure on the development of canine atopic dermatitis.

Although 100% of animals have innate immunity, only 5% of animals have acquired immunity.

Note: counted as animal species
Dr. Elie Mechnikov: Discoverer of lactobacillus, author of “macrophage”

Extensive phylogenetic work: proposal of phagocyte theory

Macrophages play an important role in the body’s defenses (for all multicellular animals)
Distribution of tissue macrophages and their physiological roles

- Defence against infection
  - Removal of:
    - amyloid
    - aging red blood cells
    - dead white blood cells

- Metabolic control
  - Regulation of:
    - iron metabolism
    - cholesterol
    - steroid
    - hormone
    - pregnancy

- Wound healing
  - Reproduction of:
    - skin
    - bone
    - liver
    - peripheral nerve
Macrophages are activated in stages

- **Preparatory stage for removal of foreign bodies**
  - An example:
    - Phagocytic capacity ↑
    - Preparatory stage for removal of foreign bodies

- **Activated macrophage (Triggering stage)**
  - An example:
    - Active oxygen ↑
    - Inflammatory cytokine ↑

**Steady-state macrophage (Resident stage)**

**Priming stage**

**Bacteria**

**Interferon**
What is LPS?

- LPS is a substance contained in bacteria, which greatly enhances self-healing power.
- LPS is $1,000 - 10,000$ times as effective as β-glucans, which are already known to be effective ingredients in mushrooms.
Utilization of micro-organisms
Past (lactobacillus) and future (gram-negative bacteria)

- **Gram-positive bacteria**
  - (bacteria with good images)
  - *Lactobacillus, bacillus subtilis*, etc.
  - Yogurt, pickles, natto, probiotics

- **Gram-negative bacteria**
  - (bacteria with bad images)
  - *E. coli*, *Pantoea agglomerans*, *acetic bacteria*, *Xanthomonas*, *Zymomonas*, etc.
  - Kefir yogurt, nata de coco, vinegar, probiotics

13
Receptor and intracellular signals of LPS

The amount for macrophages activation

β-glucan 1,000–10,000:1 LPS

β-1,3 glucan
Peptidoglycan

LPS bind in protein

TLR-2
Receptor

MD2

CD14

Dectin

Nucleus

NFkB

IRF

Interferon
(antiviral activity)

Interferon

Macrophage activation
(antibacterial activity)
Screening macrophage activating substances from foods by oral or percutaneous route

1. From foods
2. Waste products

Gluten, starch

Water extract of wheat flour contains Macrophage activating substance

What is the active substance?

Wheat seeds from Canada, USA, Australia, Japan were analyzed concomitant bacteria.

Lipopolysaccharide (LPS)

Pantoea agglomerans
A Gram-negative bacteria

**Relative mRNA expression**

- **IL-4 and IL-12:** No corelation with responses
- **IL-10:** increasing mRNA in responded dogs
Pine bark extract

- Extracted from French maritime pines with an average age of 20-25 years from Bordeaux, France.
- Compared with other types of pine, French maritime pine bark is very thick and contains more than 40 kinds of antioxidants polyphenols.
Pine Bark Polyphenolol Features

1. Excellent antioxidant action
2. Improves circulation
3. Stabilizes blood sugar
4. Powerful Anti-inflammatory action
5. Enhances the skin
2. Clinical Trial Data

Hair-growth promotion effect on bald spots:
Thirteen animals treated with combination of LPS and pine polyphenol
Details of trials

• **Methods and materials**
  – Evaluation of hair-growth effect on alopecia in dogs, cats, rabbits, and hamsters

• **Test materials**
  – “LPS tablet” and “Pine polyphenol tablet”

• **Test methods and duration**
  – Administration of foods and drugs was not regulated. Combination with other supplements was ruled out. Animals were assigned to receive either an LPS tablet alone, or to receive both LPS and pine polyphenol tablets.
  – Test duration was 30 days (in principle).

• **Test results**

<table>
<thead>
<tr>
<th></th>
<th>Hair growth</th>
<th>Number of animals</th>
<th>Rate of hair growth response</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LPS alone (n=24)</strong></td>
<td>None</td>
<td>14</td>
<td>58.3%</td>
</tr>
<tr>
<td></td>
<td>Observed</td>
<td><strong>10</strong></td>
<td><strong>41.7%</strong></td>
</tr>
<tr>
<td><strong>LPS + Pine polyphenol (n=13)</strong></td>
<td>None</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Observed</td>
<td><strong>13</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>
## List of 13 animals treated with combination of LPS and pine polyphenol

<table>
<thead>
<tr>
<th>Breed</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Body weight (kg)</th>
<th>Concomitant drugs</th>
<th>Dosage LPS</th>
<th>Concomitant drugs Pine polyphenol</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. dachshund</td>
<td>♀</td>
<td>2</td>
<td>3.5</td>
<td>None</td>
<td>2</td>
<td>4</td>
<td>Allergic dermatitis Hair regrew after 12 weeks.</td>
</tr>
<tr>
<td>M. dachshund</td>
<td>♀</td>
<td>10</td>
<td>4.2</td>
<td>Used</td>
<td>2</td>
<td>4</td>
<td>Atopic dermatitis Hair regrew after 6 weeks.</td>
</tr>
<tr>
<td>Border collie</td>
<td>♀</td>
<td>7</td>
<td>20</td>
<td>Used</td>
<td>4</td>
<td>4</td>
<td>Dry dermatitis (systemic) A steroid drug was used once.</td>
</tr>
<tr>
<td>T. Poodle</td>
<td>♀</td>
<td>3</td>
<td>2.5</td>
<td>None</td>
<td>2</td>
<td>2</td>
<td>Allergic dermatitis Deep-colored hair regrew after 7 weeks.</td>
</tr>
<tr>
<td>T. Poodle</td>
<td>♀</td>
<td>3</td>
<td>3.1</td>
<td>None</td>
<td>2</td>
<td>2</td>
<td>Stress-induced lick dermatitis Hair regrew fully after 3 weeks.</td>
</tr>
<tr>
<td>T. Poodle</td>
<td>♂</td>
<td>11</td>
<td>3.1</td>
<td>None</td>
<td>2</td>
<td>2</td>
<td>Dermatitis due to subcutaneous infection Hair regrew after 4 weeks.</td>
</tr>
<tr>
<td>T. Poodle</td>
<td>♂</td>
<td>1</td>
<td>3.9</td>
<td>None</td>
<td>2</td>
<td>2</td>
<td>Stress-induced hair loss Hair regrew after 3 weeks.</td>
</tr>
<tr>
<td>Cairn terrier</td>
<td>♂</td>
<td>12</td>
<td>8.2</td>
<td>None</td>
<td>3</td>
<td>3</td>
<td>Unexplained hair loss Itching and hair loss were relieved after 4 weeks.</td>
</tr>
<tr>
<td>Chihuahua</td>
<td>♀</td>
<td>5</td>
<td>3.2</td>
<td>Used</td>
<td>2</td>
<td>2</td>
<td>Allergic dermatitis (Convenia was used for pyoderma)</td>
</tr>
<tr>
<td>Mix-breed cat</td>
<td>♀</td>
<td>7 (estimated)</td>
<td>2.8</td>
<td>None</td>
<td>1</td>
<td>1</td>
<td>Unexplained hair loss Hair regrew after 4 weeks.</td>
</tr>
<tr>
<td>Mix-breed cat</td>
<td>♀</td>
<td>12</td>
<td>2.7</td>
<td>None</td>
<td>2</td>
<td>2</td>
<td>Unexplained hair loss Hair regrew after 4 weeks.</td>
</tr>
<tr>
<td>Hamster</td>
<td>♀</td>
<td>1</td>
<td>Not weighed</td>
<td>None</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>Unexplained hair loss Hair regrew after 4 weeks.</td>
</tr>
<tr>
<td>Rabbit</td>
<td>♀</td>
<td>5</td>
<td>2.5</td>
<td>None</td>
<td>1</td>
<td>1</td>
<td>Self-inflicted hair loss Hair regrew after 9 weeks.</td>
</tr>
</tbody>
</table>
3. Putative Mechanisms of Action
Putative mechanisms of action

- **LPS**
  1. Macrophages are turned into priming stage and inhibit induction of catagen-inducing cytokine.
  2. Combination of mechanisms generates effect.

- **Pine polyphenol**
  1. Antioxidant effect
  2. Blood circulation promoting effect
Animals also smile.

Thank you for listening.

Hiroshi Okawa
Representative Director of Scarecrow Inc., Tokyo, Japan
contact@scarecrow-inc.co.jp