Specific Egg Yolk Antibody (Ovalgen PG) as a Novel Supportive Immunotherapy for Periodontitis

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Agenda

- About IgY antibody
- Porphyromonas gingivalis & gingipain
- In-vitro tests
- Studies on animals
- Clinical trials on volunteers
IgY antibody

Yolk immunoglobulin = IgY
Advantages of IgY compared to IgG from mammals

- Higher avidity compared to IgG (better effect)
- Does not bind to Fc receptor and not react with human complement system
- Safe (present in human food every day)
- High hygienic farming conditions of poultry
Bacteria (Treponema denticola)  T. denticola and IgY
IgY products for oral care purpose

- **Ovalgen® DC**: IgY for controlling dental caries
- **Ovalgen® PG**: IgY for controlling periodontitis
- **Ovalgen® CA**: IgY for controlling Candidiasis
Clinical trial with Ovalgen DC: S. mutans in saliva

Before

After
Clinical trial with Ovalgen DC: dental plaques

Before

After
Oral care products with Ovalgen

Hakira (BSS) Tablet

Oraclear (UM) Tablet

Hakira (BSS) Toothpaste

Hareguard (BSS) Tablet

IgYGate DC-F

IgYGate DC-PG
Part 2: Porphyromonas gingivalis & gingipain

*Porphyromonas gingivalis*
- Gram negative
- Anaerobic
- Black shiny and smooth colony on blood agar

Gingipain is the main pathogenic factor of P. gingivalis
Functions of Gingipain

- Causes damages to various tissues and systems (intercellular proteins, immune cells & cytokines, fibrin & fibrinogen, vv).

- Helps P. gingivalis invade into host cells (cell invasion).

- Helps P. gingivalis to agglutinate with other bacteria forming biofilm.

- Absorbs into blood causing systemic effects (heart diseases, early birth, etc)
Periodontitis is associated with systemic diseases

**Stroke**
Increase the risk of stroke by over 50% in adults.

**Diabetes**
Periodontal (gum) disease disrupts glycemic control.

**Heart disease**
Risk of fatal heart disease is **twice as high** for individuals with severe gum disease.

**Preterm birth**
Pregnant women with severe gum disease are **seven times more likely** to have a premature baby.

Sources: National Institute of Dental and Craniomaxillofacial Research and American Heart Association
Preparation of IgY against gingipains

1. Collect yolk
2. Purify IgY
3. IgY (Ovalgen PG)
## Results: Cell damage inhibition

<table>
<thead>
<tr>
<th>Sample</th>
<th>total No. of Cells</th>
<th>Protection after P. gingivalis treatment:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>alive cells</td>
</tr>
<tr>
<td>PG only</td>
<td>15.8</td>
<td>2.2</td>
</tr>
<tr>
<td>PG + Control IgY</td>
<td>14.8</td>
<td>4.1</td>
</tr>
<tr>
<td>PG + Ovalgen PG</td>
<td>15.9</td>
<td>13.7</td>
</tr>
</tbody>
</table>

(number of alive cells x $10^4$)
Part 4: Study on animals
Trial on Sprague-Dawley
(by Prof. Hamada, Kanagawa Dental College)

A: non-infected control  
B: infected, non-treated  
C: infected, treated with cont IgY  
D: infected, treated with IgY-PG

Source: AAP/JSP 2010 Annual Meeting
Trial on dogs
Effect of passive immunization by anti-gingipain IgY on periodontal health of dogs

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Abstract

Anti-gingipain IgY (IgY-GP), known as hyperimmune γ-livetin from egg yolk, inhibits the enzyme activity, growth and adherence of Porphyromonas gingivalis to gingival epithelial cells. Our objective was to evaluate the efficacy of IgY-GP on periodontal health of dogs. IgY-GP was prepared from the egg yolk of hens immunized with the gingipain from Porphyromonas gingivalis ATCC 33277. Two in vivo trial models were conducted on 15 adult dogs with periodontitis by giving IgY-GP-supplemented dog feed for 8 weeks and direct application of the IgY in dental ointment to the active lesions of periodontitis in humans2 and its subgingival implantation in mice,3 rats4 and non-human primates5 is associated with initiation and progression of the disease.

Virulence of P. gingivalis is associated with the proteolytic enzymes gingipains6 that are produced as secreted or membrane-associated forms by the bacterium.7 Gingipains are cysteine proteinases that can degrade key components of the immune system.8 In addition, gingipains are important for the bacterium to proliferate and survive in the periodontal pockets.9 These facts suggest that gingipains are the most promising target for vaccination against periodontitis and related systemic diseases.

Immunotherapy by specific chicken antibodies (IgY) has been used with mixed successes against infectious diseases of viral, bacterial and fungal origin on both humans and animals.10-12 Peroral administration with IgY is an attractive approach because IgY does not activate mammalian complement or interact with mammalian Fc receptors that could mediate inflammatory response in the gastrointestinal tract.13 In recent papers we have reported that anti-gingipain IgY had preventive effect against periodontitis in human patients.14 In the present study we examined if the same IgY has effect against periodontal diseases in companion animals when used in different applications.

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Key words: Gingipain, IgY, Periodontitis, Pet, Porphyromonas gingivalis.

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Conflict of interest: the authors report no conflicts of interest.

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Dog: Beagle (Average Age = 7 yrs, Average BW = 8 kg)

Groups:
- Test Group (n = 10): 0.62 mg IgY/dog/day (mixed with dry food)
- Control Group (n = 10): Control food

Evaluation: 0 W, 4W, 8W

Parameter:
- Inflammation Score: (Foul breath, Gum congestion, Bleeding of gums, Gum ulcer, Periodontal ligament inflammation)
Breath odor and gum congestion

Foul breath odor score

Gum congestion score
Bleeding gums and gum inflammation score

Effect of Globigen PG on Gingival bleeding of Dog

Effect on Gum inflammation of Dog

Dog Groups

Gingival bleeding score

Dog Groups

Gum inflammation score

p < 0.01

* * *
Part 5: clinical trials on volunteers
Clinical test: effect of Ovalgen PG in periodontitis patients
Effect of egg yolk antibody against *Porphyromonas gingivalis* gingipains in periodontitis patients

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Abstract: *Porphyromonas gingivalis* gingipains is suspected to be one of the most important causative agents of periodontitis. We postulated that the inhibition of gingipains may reduce the pathogenic nature of *P. gingivalis*. Anti-*P. gingivalis* egg yolk antibody (IgY-GP) was isolated from the yolks of hens immunized with purified gingipains. We applied IgY-GP gel subgingivally in periodontitis patients who harbored periodontitis. (J. Oral Sci. 49, -, 2007)

Keywords: gingipains; IgY; periodontitis; *Porphyromonas gingivalis*.

Introduction

Periodontitis is an inflammatory disorder that results in
Protocol

• **Test sample:**
  - Neutral gel containing 20mg Ovalgen PG/syringe

• **Subject:** 5 periodontitis patients

• **Administration:** One dose of gel applied into pockets after scaling and root planing (SRP). Control pockets only treated by scaling and SRP.

• **Examination parameters:** (check before and 4 weeks after)
  1. Oral examination: Probing depth (PD), Bleeding on probing (BOP).
  2. Bacteria: number of P. gingivalis and total bacteria in periodontal pockets (by real-time PCR).
Control teeth
(Right half of mouth)

Test teeth
(Left half of mouth)

Root Canal Syringe filled with OVALGEN PG gel
Oral examination

**Probing Depth**

- **Control**
- **Ovalgen PG**

*P* < 0.05

**BOP**

*P* < 0.05

(probing depth and BOP with control and Ovalgen PG treatment)
Result: ratio of *P. gingivalis* in periodontal pocket
Clinical trial 2: use of tablets containing Ovalgen PG

- Samples: tablets containing Ovalgen PG or control IgY
- Volunteers: 34 periodontitis patients (test: 18, placebo: 16)
- Method: randomized double blind placebo controlled
  Volunteers used tablets for 12 weeks (3 times/day) after SRP treatment.
- Evaluation:
  1. Pocket depth (PD), bleeding on probing (BOP).
  2. Ratio of P. gingivalis in pockets (by real-time PCR).
## Results

<table>
<thead>
<tr>
<th>Test</th>
<th>BOP (%)</th>
<th>PD (mm)</th>
<th>P.g level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>31.5 ± 19.4</td>
<td>2.3 ± 0.6</td>
<td>2.2 ± 4.6</td>
</tr>
<tr>
<td>12W</td>
<td>25.3 ± 16.9</td>
<td>1.1 ± 0.6*</td>
<td>1.0 ± 3.1*</td>
</tr>
<tr>
<td>Baseline</td>
<td>38.9 ± 21.9</td>
<td>2.4 ± 0.6</td>
<td>1.4 ± 2.0</td>
</tr>
<tr>
<td>12W</td>
<td>32.3 ± 21.6</td>
<td>2.3 ± 0.6</td>
<td>1.0 ± 2.1</td>
</tr>
</tbody>
</table>

*P<0.05

Research Forum Poster Session *in* 2009 American Academy of Periodontology Annual Meeting, Boston, USA
Summary

- Use of anti-gingipain IgY combined with SRP helps improve PD, BOP.

- anti-gingipain IgY (Ovalgen PG) can be used to enhance treatment effect of SRP and prevent relapse.
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