Cyclo-oxygenase 2 (COX-2) as a Target for the Inhibition of the Hypertrophic Effects of Endothelin-1 by Ginseng

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Cardiac Hypertrophy is a Risk Factor for Heart Failure

Hypertrophic stimuli

- G-protein-coupled receptors

Transcription Factors

↑ hypertrophic gene

Compensatory mechanisms

Decompensatory mechanisms

Heart failure

 Symptoms of heart failure
Cardiac Hypertrophy Mechanisms
ET-1 and Its Receptors

ET1 gene promoter

Prepro-ET1 mRNA

Prepro-ET1 → pro-ET1

ECE-1

ET1

Endothelium

Smooth muscle

Cardiacmyocyte

ET1

ET1

Vasoconstriction

Vasodilation

ET_A

ET_B

ET_A

ET_B

Vasoconstriction

Vasodilation

Pos. inotropy
Neg. chronotropy
Hypertrophy

Pos. lusitropy
Pos. chronotropy
What are the Benefits of Ginseng?

- A nutritious herb as an energy tonic and folk medicine in China
- Increase in physical performance
- Dealing with mental & emotional stress
- Preventing the growth of some cancer cells
- Benefit for the treatment of diabetes
Beneficial roles of Ginseng in Cardiac Hypertrophy and Heart Failure


Possible Involvement of COX-2 in Cardiac Hypertrophy

Phospholipids
\[ \downarrow \text{PLA}_2 \]
Arachidonic acid
\[ \downarrow \text{COX-2} \]
\[ \text{Celecoxib (Celebrex®)} \]
\[ \downarrow \]
\[ \text{PGG}_2 \]
\[ \downarrow \text{peroxidase} \]
\[ \text{PGH}_2 \]
\[ \downarrow \text{prostaglandin synthases} \]
\[ \text{PGI}_2 \quad \text{PGF}_{2\alpha} \quad \text{PGE}_2 \quad \text{PGD}_2 \]
Hypertrophy
Evidence Linking COX-2 with Cardiovascular Diseases


Hypothesis

Inhibition of ET-1-induced COX-2 upregulation mediates the antihypertrophic effect of ginseng
Specific Aims

- To determine the effect of ET-1 on COX-2 expression and activity

- To determine the effect of COX-2 inhibition on the hypertrophic effect of ET-1

- To determine whether the antihypertrophic effect of ginseng is associated with prevention of COX-2 induction
Neonatal ventricular myocytes (1-5 days SD rats)

Plating (1X10^4/cm²) → 24 h treatment

**Experimental Design**

**Hypertrophy Assessment**
- Cell surface area
- ANP

**Mechanism studies**
- RNA isolation
- Protein isolation
- Western blot
  - Activity
  - COX-2
- Immunostaining
  - NF-κB
  - Nuclear Translocation
ET1-induced COX-2 upregulation is inhibited by ginseng

mRNA | protein | activity

- COX-2
- β-Actin

<table>
<thead>
<tr>
<th>COX-2/18s rRNA</th>
<th>COX-2/β-Actin</th>
<th>COX-2 activity (nmol/min/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>ET</td>
<td>ET+Ginseng</td>
</tr>
<tr>
<td>1</td>
<td>2.5</td>
<td>1</td>
</tr>
<tr>
<td>1.5</td>
<td>2.8</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>3.5</td>
<td>1.5</td>
</tr>
<tr>
<td>5</td>
<td>4.5</td>
<td>3.0</td>
</tr>
<tr>
<td>*</td>
<td>**</td>
<td>*</td>
</tr>
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</table>

N=8. *p<0.05 from control.  #p<0.05 from ET-1

C: Control
ET: ET-1(10nM)
Ginseng(10ug/ml)
ET-1 induced Hypertrophy is Prevented by the COX-2 Inhibitor Celecoxib

C: control  
ET: ET-1  
Cele: Celecoxib selective COX-2 inhibitor (10uM)

N=8. *p<0.05 from control.  
#p<0.05 from ET-1
Potential role of NF-κB in mediating COX-2 induction
Ginseng Inhibits COX-2 Upregulation via NF-κB Inhibition

ET-1(10nM)
GS(μg/ml)
NFI: NF-κB inhibitor peptide 1μM

N=8. * p<0.05 from control.
#p<0.05 from ET-1.
Ginseng Prevents ET-1-Induced NF-κB Nuclear Translocation

Control                          ET1                    ET1+Ginseng

<table>
<thead>
<tr>
<th>Nuclear Fluorescence Intensity</th>
<th>C</th>
<th>ET1</th>
<th>ET1+GS</th>
</tr>
</thead>
</table>

*Statistically significant difference compared to ET1

#Statistically significant difference compared to C
Potential pharmacodynamic interaction between ginseng and the ET$_A$ receptor antagonist BQ123
Significant roles of a combination of ginseng and ETA receptor antagonist in ET-1 induced cardiac hypertrophy

Cardiac hypertrophy is the risk factor for heart failure. There is substantial evidence that the formation and release of ET-1 as well as ET receptor expression is enhanced in both humans and animal models with chronic heart failure. Locally produced ET-1 greatly increases the contractility of cardiac muscle, therefore exerting an immediate beneficial effect on damaged tissue. However, in the long term, ET-1 induces myocardial hypertrophy which is associated to a maladaptive effect on myocardial structure and function, thereby leading to fatal events.

In fact, an important number of studies have reported beneficial effects of the use of ET receptor antagonists, mostly targeting ETA receptor, in chronic heart failure as evidenced by a reduced infarct size, and improved reperfusion coronary flow. In view of these results, the therapeutic potential of ET receptor antagonists in human heart failure has been tested in randomized clinical trials. However, most of these studies failed to show a clear improvement in the clinical status of patients, and very frequently, trials involving ET receptor antagonists had to be stopped prematurely due to disease worsening or liver toxicity. Therefore, the potential benefit of ET receptor antagonists cast doubt on the usefulness of these drugs for the treatment of heart failure patients and still needs to be defined.

References:
From the clinical point of view, novel therapeutic strategies are need to reduce the clinical toxicity of ET\textsubscript{A} receptor antagonist in the treatment of heart failure. Therefore, we investigated the effects of the combination of ginseng with ET\textsubscript{A} receptor antagonist in ET-1-induced cardiac hypertrophy by suboptimal doses of ET\textsubscript{A} receptor antagonist.
Both ginseng and BQ123 prevent ET-1 induced cardiomyocyte hypertrophy

Both ginseng and BQ123 prevent ET-1 induced cardiomyocyte hypertrophy

Both ginseng and BQ123 prevent ET-1 induced cardiomyocyte hypertrophy

N=6  * p<0.05 from control.
    # P<0.05 from ET-1.
Ginseng and BQ123 prevent ET-1 induced ANP expression

N=8. *p<0.05 from control.

#p<0.05 from ET-1
Additive effects of ginseng in combination with BQ123 against ET1-induced cardiomyocyte hypertrophy

**Cell surface area (μm²)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mean (μm²)</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont</td>
<td>800 ± 100</td>
<td></td>
</tr>
<tr>
<td>ET</td>
<td>1200 ± 150</td>
<td></td>
</tr>
<tr>
<td>ET+GS(1)</td>
<td>1000 ± 200</td>
<td></td>
</tr>
<tr>
<td>ET+BQ(10)</td>
<td>800 ± 150</td>
<td></td>
</tr>
<tr>
<td>ET+GS(1)+BQ(10)</td>
<td>600 ± 100</td>
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</table>

**ANP/18s rRNA**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mean (ARPM)</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont</td>
<td>2 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>ET</td>
<td>4 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>ET+GS(1)</td>
<td>4 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>ET+BQ(10)</td>
<td>2 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>ET+GS(1)+BQ(10)</td>
<td>1 ± 0.1</td>
<td></td>
</tr>
</tbody>
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* p<0.05 from control. # p<0.05 from ET-1.

N=6.
ET1-induced COX-2 upregulation is inhibited by the combination of ginseng and BQ123 at suboptimal concentrations.

### mRNA
- COX-2/18s rRNA

### Protein
- COX-2
- β-Actin

### Activity
- COX-2 activity (nmol/min/ml)

**Legend:**
- ET1: ET-1 (10nM)
- GS: GS (μg/ml)
- BQ123: BQ123 (nM)

**Graphs:**
- Bars represent the relative expression levels of COX-2 mRNA, protein, and activity with different treatments.
- * p<0.05 from control.
- # p<0.05 from ET1.

**N=8.**
ET1-induced NF-κB gene expression is inhibited by the combination of ginseng and BQ123 at suboptimal concentrations.

**Graph:***

- **Control**
- **ET-1**
- **ET-1+GS (1μg/ml)**
- **ET-1+BQ(10nM)**
- **ET-1+GS1+BQ10**

**Legend:***
- ET-1 (10nM)
- GS (μg/ml)
- BQ123 (nM)

**Notes:***
- N=8. *p<0.05 from control.
- #p<0.05 from ET-1
ET1-induced NF-κB nuclear translocation is inhibited by the combination of ginseng and BQ123 at suboptimal concentrations.
The proposed mechanisms of ginseng and BQ123 against ET-1 – induced COX-2 gene expression is mediated by NF-κB
Summary of Results

- ET1-induced cardiomyocyte hypertrophy was prevented by ginseng or ET$_A$ receptor blocker.

- The antihypertrophic effect of either agent was associated with diminished activation of NF-κB and COX-2 gene expression and activity.

- Ginseng or BQ123 alone at suboptimal concentrations did not inhibit ET1-induced cardiomyocyte hypertrophy and also had no effect on ET1-induced activation of NF-κB and COX-2.

- Ginseng and BQ123 demonstrate additive effects when used together at suboptimal concentrations by attenuating ET-1 induced hypertrophy as well as NF-κB and COX-2 activation.
Conclusion

Prevention of ET-1–induced hypertrophy by ginseng is mediated by attenuation of COX-2 upregulation through a NF-kB-dependent mechanisms

Suboptimal concentrations of ginseng and the ET<sub>A</sub> receptor antagonist BQ123 produce additive effects against ET-1 induced hypertrophy and COX-2 induction
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