The immunomodulator ginsan induces resistance to experimental sepsis by inhibiting Toll-like receptor mediated inflammatory signals


Ji-Yeon Ahn1,2, In-Soo Choi3, Ji-Young Shim1, Eun-Kyung Yun1, Yeon-Sook Yun1, Gajin Jeong2 and Jie-Young Song1
Ginseng contains multiple phytochemicals:

Two major classes:
- Ginsenosides
- Polysaccharides

Organic extract contains mostly ginsenosides

Aqueous extract contains less ginsenosides and more polysaccharides
- What are ginsenosides?
- What are polysaccharides?
Basic structure of ginsenosides:

Glycoside = aglycone + sugar chain
(glucose, maltose, fructose, saccharose attached at C3, C6 and C20)

3 major groups depending on their aglycones:

Group I - protopanaxadiol type
Group II - protopanaxatriol type
These are C-27 sterols (with a dammarane skeleton aglycone)

Group III - oleanolic acid-type triterpenoids.
- Water-soluble and acidic polysaccharides
- Ginsan, acidic polysaccharide with a M.W. of 150,000
- Cold-fX: poly-furanosyl-pyranosyl-saccharides
<table>
<thead>
<tr>
<th>Ginsenosides</th>
<th>PS</th>
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<tbody>
<tr>
<td>-immunomodulatory</td>
<td>+</td>
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<tr>
<td>TNF</td>
<td>TNF</td>
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<tr>
<td>IL-1, IL-2, TNF</td>
<td></td>
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<tr>
<td>-anti-inflammatory</td>
<td>?</td>
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<tr>
<td>-radioprotective</td>
<td>+</td>
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<tr>
<td>-antioxidative</td>
<td>+</td>
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<tr>
<td>reduce ROS and increase anti-oxidant levels</td>
<td>+</td>
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<tr>
<td>-anti-tumor/ metastasis</td>
<td>+</td>
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<tr>
<td>-angiogenesis</td>
<td>+/-</td>
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Sepesis:

- Microbial infection in blood $\rightarrow$ excessive inflammatory response $\rightarrow$ systemic organ failure

- Proinflammatory cytokines as mediators
  - TNF, IL-1, IL-6
  - IL-12 + IL-18 $\rightarrow$ are important because they produce $\rightarrow$ IFN-gamma
  - IFN + TNF $\rightarrow$ synergistic effects in LPS effect

- Gram-ve bacteria E. Coli $\rightarrow$ LPS from cell wall: mediating agent

- Gram +ve bacteria: S. aureus $\rightarrow$ lipoteichoic acid, peptidoglycan from cell wall
Toll like-receptors:
-Single membrane-spanning non-catalytic receptors, Innate immune system,
-recognize threat, recognize molecules shared by pathogens but different from host molecules

Function as dimers
Need co-receptors

Adaptor proteins

Require kinases activation for signaling and modulation of gene expression

ligands e.g. lipo-peptides, glycolipids, lipoteichoic acids, LPS, HSP70, zymosan, single or double-stranded RNA, fibrinogen, small xenobiotics
Rationale:

- Reducing a particular cytokine not effective in sepsis
- Ginsan as effective Biological Response Modifier
- Stimulate NK & T cells, induce cytokines, induce tumoricidal & antimicrobial activity in macrophages.
- Stimulate NO production in macrophages-in vitro $\rightarrow$ antisepticemic activity… by extension!
- But these mediators also enhance septic symptoms!

- Question: how does ginseng modulate plasma cytokine profile in septic animals and whether there are other mechanisms that protect animals from sepsis
Results

1. Ginsan (IV) 24 hr pretreatment protected mice from acute sepsis (3 models)
   - S. aureus induced lethality (10% to 88% survival)
   - E. coli induced lethality
   - CLP induced lethality

25 ug/kg effective, quite low dose

2. Ginsan enhanced clearance of bacteria from blood, spleen and kidney

- Normal mice $\rightarrow$ isolated PM $\rightarrow$ incubated with ginsan in vitro 3 hr $\rightarrow$ partially killed labeled bacteria $\rightarrow$ analysed by FACS for uptake of bacteria by macrophages $\rightarrow$ index of phagocytosis.

- But PM isolated from 24 hr pretreatment with ginsan with or without infection showed small if any increase of phagocytosis. Major weakness of lack of in vivo effect!

- May be PM is not the major site of bacterial clearance
Ginsan enhances phagocytosis in S. aureus-infected macrophages. (A) Phagocytic activity was evaluated in PM isolated from intact mice and incubated with ginsan for 3 h. (B) PM were obtained from non-treated (dotted line) and S. aureus-infected mice treated with or without ginsan (25 lg/kg, bold and solid lines, respectively), and were then stimulated with heat-killed S. aureus for 30 min at 37C.
4. Ginsan attenuates pro- and anti-inflammatory cytokine production in S. aureus-infected mice. Serum cytokine levels were determined at 0, 2, 4, 8, 11, and 24 h after the challenge with 1.5 × 10⁸ CFU S. aureus

(No effect on Th2 cytokines IL-2 and IL-4)  
Ginsan also reduced anti-inflammatory IL-10

Note: -Cytokines are not detectable in control animals and ginsan did not stimulate any!  
-Not consistent with hypothesis.
5. Ginsan suppresses the expression of TLR and the adaptor MyD88 molecule in PM activated by S. aureus.

In vitro only
PM isolated and treated with ginsan (0.1 ug/ml) for 6 hrs.
Cells were washed, treated heat-killed S. aureus for 6 hrs.
Measured by RT-PCR for RNA transcripts for TLR2, 4, 9 and MyD88
6. Ginsan inhibits S. aureus-induced MAPK and NF-kB activation in PM. PM were pretreated with ginsan (0.1 ug/ml) for 6 h and were subsequently treated with heat-killed S. aureus for 40 min. (A) MAPK phosphorylation was detected using Western blot analysis with antibodies specific for JNK1/2, P38, and ERK1/2.

(B) The concentration of NF-kB in nuclear extracts was determined using electrophoretic mobility shift assay.

D-R study to look for correlation?
Points for discussion

1. Low effective dose: 25 ug/kg
   Given by IV!

2. Explanation of changes in pro-inflammatory and anti-inflammatory cytokines not very convincing.

   "Ginsan eventually restore the balance between the proinflammatory and anti-inflammatory arms of the cytokine network in sepsis. Different time of infection is critical to the profiles but they did not show that.

   (No effect on Th2 cytokines IL-2 and IL-4

   Ginsan also reduced anti-inflammatory IL-10

   Note: -Cytokines are not detectable in control animals and ginsan did not stimulate any!

   -Not consistent with hypothesis."
Back to the rationale:

- Stimulate NO production in macrophages-in vitro → antisepticemic activity… by extension!
- But these mediators also enhance septic symptoms!
- Question: how does ginseng modulate plasma cytokine profile in septic animals and whether there are other mechanisms that protect animals from sepsis

Discussion:

- Unpublished data from author:
  Ginsan stimulated TLR in normal macrophages (not shown in present study) but down regulate them in septic macrophages….suggest ginsan could induce tolerance against septic challenges.
- Other: sublethal dose LPS pretreatment protect subsequent LPS induced lethality by reducing proinflammatory cytokines
- Major weakness; not show effect of ginsan in present study to test the hypothesis
Tripterygium Wifordii Extracts [EA & PS] on LPS-induced NO production in macrophages

EA also inhibited COX-2 m-RNA expression and PGE2 production

Others showed inhibition of cytokines production