The link between shockwaves and innate immunity – the main working mechanism?

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**Effects**

**Extracorporeal Shock Wave Therapy Induces Therapeutic Lymphangiogenesis in a Rat Model of Secondary Lymphoedema**

F. Serizawa a, K. Ito b, M. Matsubara c, A. Sato a, b, H. Shimokawa b, S. Satomi a

**Shock Wave-Pretreated Bone Marrow Cells Further Improve Left Ventricular Function After Myocardial Infarction in Rabbits**

Jiunn-Jye Sheu, a Chen-Kwan Sun, a Li-Teh Chang, b Hsiu-Yu Fang, b Sheng-Ying Chung, b Sarah Chu a, b Morgan Fu, a Fan-Yen Lee, a Ying-Hsien Kao, b Shuey-Fat Ko, b Ching-Jen Wang, a Chia-Hung Yen, b Steve Leu, a and Hon-Kan Yip, a Kaohsiung and Pingtung, Taiwan, ROC

**VEGF Modulates Angiogenesis and Osteogenesis in Shockwave-Promoted Fracture Healing in Rabbits**

Ching-Jen Wang, M.D. a, b, † Ko-Er Huang, M.D. ‡ Yi-Chih Sun, B.A. ‡ Ya-Ju Yang, B.A. ‡ Jih-Yang Ko, M.D. a, b, † Lin-Hsin Wong, M.D., a, b and Peng-Sheng Wang, Ph.D. †

*Department of Orthopedics, †Department of Otolaryngology, and ‡Department of Medical Research, Chang Gung University College of Medicine, Chang Gung Memorial Hospital-Kaohsiung Medical Center Kaohsiung, Taiwan

**Effect of shock wave number on renal oxidative stress and inflammation**

Daniel L. Clark, Bret A. Connors, Andrew P. Evan, Rajash K. Handa and Sujuan Gao*

Department of Anatomy and Cell Biology, and *Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, USA

Accepted for publication 4 January 2010

**Extracorporeal shock waves: From lithotripsy to anti-inflammatory action by NO production**

Sofia Mariotto a, 1, Elisabetta Cavalieri a, 1, Ernesto Amelio b, Anna Rosa Ciampa c, Alessandra Carcereri de Prati a, Ernst Marlinghaus c, Sergio Russo d, Hisanori Suzuki a, 2

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† Shock Wave Unit, Hand Surgery Department, Polyclinic G.B. Rossi, Largo L.A. Scuro 10, 37134 Verona, Italy
‡ Department of Orthopaedics, Polyclinic Federico II, University of Naples, Via S. Pansini 5, 80131 Naples, Italy

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RNA
so many effects...
same control unit?
RNA content
cytosolic RNA
membrane permeability

CTR

SWT

ctr 2h.002

SWT 2h.002
known effect
What happens with RNA?
Toll-like receptor 3

Nature Reviews Immunology 2006
Pilot: TLR-3 activation in HUVECs

**TLR-3 Poly I:C stimulated**

**TLR-3 mRNA**

<table>
<thead>
<tr>
<th></th>
<th>CTRL</th>
<th>2h</th>
<th>4h</th>
<th>6h</th>
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<td>6h</td>
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Significance levels: *** at 6h
Part I

*in vitro* experiments
TLR-3 reporter cells
positive proof
TLR-3 gene knock-down with siRNA

negative proof!

read-out gene:
Part II

*in vivo* experiments
Animal model

hind limb ischemia

wild type vs. TLR-3 k.o. mice

Time Course:

HLI  3 weeks  +24h  +28h  + 4 weeks
Shock Wave therapy

• Molecular Biology
• Molecular Biology
• IHC
• IHC
• Laser Doppler
TLR-3 signalling
TLR-3 signalling – in vivo

TRIF Western in vivo ko

<table>
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<tr>
<th>Condition</th>
<th>rel. protein expression</th>
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<tr>
<td>TLR3+HL1+SWT72h</td>
<td>0</td>
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<tr>
<td>TLR3+HL72h</td>
<td>50</td>
</tr>
<tr>
<td>WT+HL1 72h</td>
<td>100</td>
</tr>
<tr>
<td>WT+HL1+SWT72h</td>
<td>150</td>
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</table>

*** P < 0.001
* P < 0.05
TLR-3 signalling – *in vivo*

**IRF3 Western in vivo ko**

<table>
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<tr>
<th>Condition</th>
<th>rel protein expression</th>
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<tbody>
<tr>
<td>TLR-3+HL+SWT 72h</td>
<td>*</td>
</tr>
<tr>
<td>TLR-3+HL 72h</td>
<td>**</td>
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<tr>
<td>WT+HL 72h</td>
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<tr>
<td>WT+HL+SWT 72h</td>
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TLR-3 signalling – in vivo

![Diagram showing TLR-3 signalling pathway]

**HLI in vivo ko Animals**

Traf 6 mRNA 72h

<table>
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<tr>
<th>Condition</th>
<th>Traf 6 mRNA (arbitrary units)</th>
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<tbody>
<tr>
<td>TLR-3+SWT 72h</td>
<td><strong>500</strong> ± 50</td>
</tr>
<tr>
<td>TLR-3+HLI 72h</td>
<td><strong>400</strong> ± 40</td>
</tr>
<tr>
<td>WT+SWT 72h</td>
<td><strong>300</strong> ± 30</td>
</tr>
<tr>
<td>WT+HLI 72h</td>
<td><strong>200</strong> ± 20</td>
</tr>
<tr>
<td>WT+HLI+SWT 72h</td>
<td><strong>100</strong> ± 10</td>
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</table>

**Significance:** ***
TLR-3 signalling – in vivo
morphological outcome
immunohistochemistry
Tissue necrosis

Necrosis Score

- TLR-3-/HLI
- TLR-3-/HLI+SWT
- Wt HLI
- Wt HLI+SWT

Day 7, Day 14, Day 21, Day 28

Ctrl, CST
angiogenesis

HLI in vivo ko Animals
KDR mRNA 72h

HLI in vivo ko Animals
PIGF mRNA 72h
### angiogenesis

<table>
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<tr>
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<th>Wt HLI+SWT</th>
<th>TLR-3 -/- HLI+SWT</th>
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<td><strong>72h</strong></td>
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laser doppler perfusion imaging
laser doppler perfusion imaging

**Laser Doppler**

- **TLR-3−/−HLI**
- **TLR-3−/−HLI+SWT**
- **Wt HLI**
- **Wt HLI+SWT**

- **Hindlimb perfusion ratio (l vs. r leg)**
  - 0.0
  - 0.2
  - 0.4
  - 0.6
  - 0.8
  - 1.0

- **Days:** Day 0, Day 7, Day 14, Day 21, Day 28

The graph shows the hindlimb perfusion ratio over time for different conditions.
Summary

Shock waves cause

- release of nucleic acids
- stimulation of the innate immune system via TLR-3
- environment for regeneration
- effects are missing in knock-down and knock-out models

- majority of SWT effects are mediated by TLR-3
Innate immunity