



Medical Pride
KUMC

EFFECTS OF LIPOPOLYSACCHARIDE ON CHANGES IN RED BLOOD CELLS IN MICE ENDOTOXEMIA MODEL

Seol-ju Park, Jei-hak Myung, Yun-hee Kim,
Sung-mook Yoo, Jae-kwan Lim, Choon-Hak Lim

Department of Anesthesiology and Pain Medicine,
Korea University Anam Hospital, Seoul, Korea



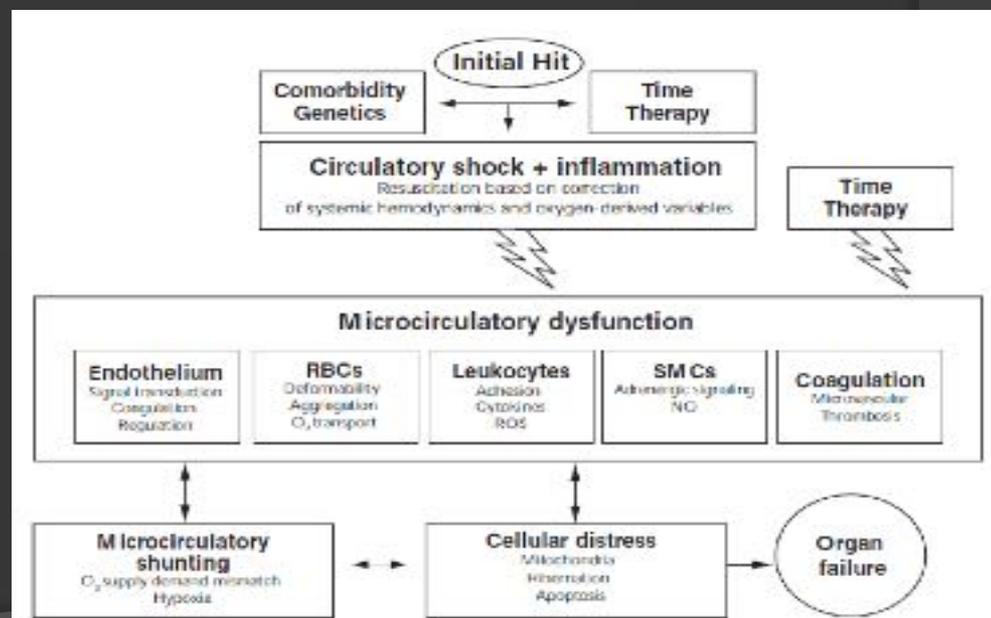
Sepsis

- a life-threatening clinical disease
- Severe sepsis and septic shock
 - associated with hospital mortality rate (28.4%)
- **Complex pathophysiological process :**
 - changes in the microcirculation
 - changes in the biochemical and physiological characteristics of the blood constituents
- Persistent microcirculation alterations
 - associated with multiple organ failure and death



Alterations in red blood cell (RBC) rheology

- Derangement in microcirculatory perfusion
- In septic patients and animals
- **Decrease of RBC deformability**
- **Increase of RBC aggregation**
- Increase of adhesiveness between WBCs, platelets, and endothelial cells





Objective

- a dynamic study to assess RBC deformability in relation to shear stress applied to the membrane
- The time course of RBC alterations : not been revealed
- The specific shear stress to detect RBC deformability : not been suggested

Evaluation of

- **changes in RBC aggregation and deformability** over 24 hour
- **specific shear stress** values for detecting RBC deformability in a mice endotoxemia model using lipopolysaccharide



Materials

- Six-week-old male BALB/c mice
- **Lipopolysaccharide (LPS)**
(Escherichia coli, serotype 0111, B4; Sigma, St.Luis, MO, USA)
- **LPS-induced sepsis group** (n = 6, LPS group)
 - injection 20 mg/kg of LPS in 0.5 mL saline intraperitoneally
- **Control group** (n = 4, saline group)
 - injection of 0.5 mL saline intraperitoneally

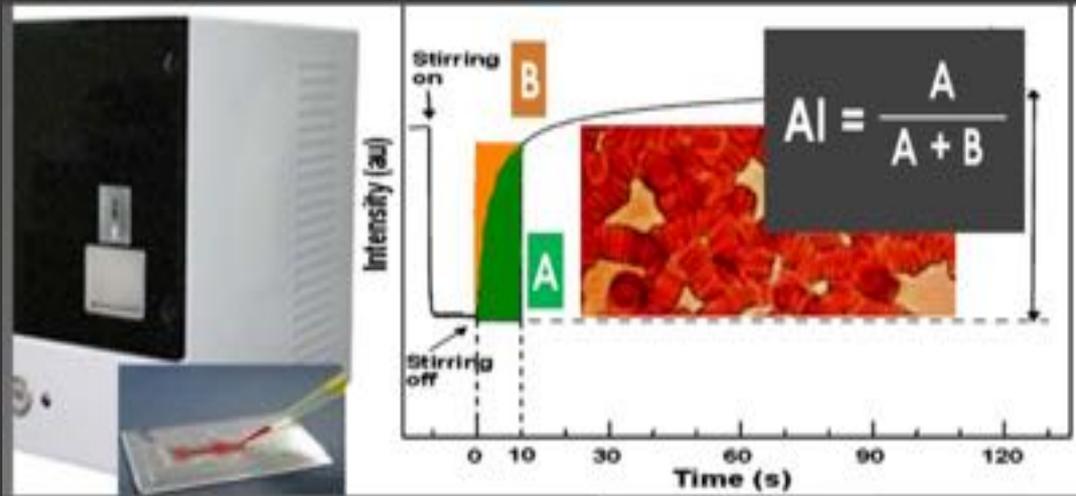


Methods

- Observation at 30 min, 2 hour and 24 hour after injection
 - compare between LPS induced group and control group
 - measure RBC aggregation and deformability
- **10 sub-experimental group (n = 6 / group)**
 - 0, 30min, 1, 2, 4, 6, 9, 12, 18 and 24 hours after the LPS injection
 - for the time course of the RBC alterations
 - measure RBC aggregation and deformability
& Serum pro-inflammatory cytokines (interleukin [IL]-6 and IL-12) levels
- Anesthetic method : intraperitoneal injection of 50 mg/kg Zoletyl.
- Blood sampling site : inferior vena cava (IVC)

Analytical procedure

○ RBC aggregation

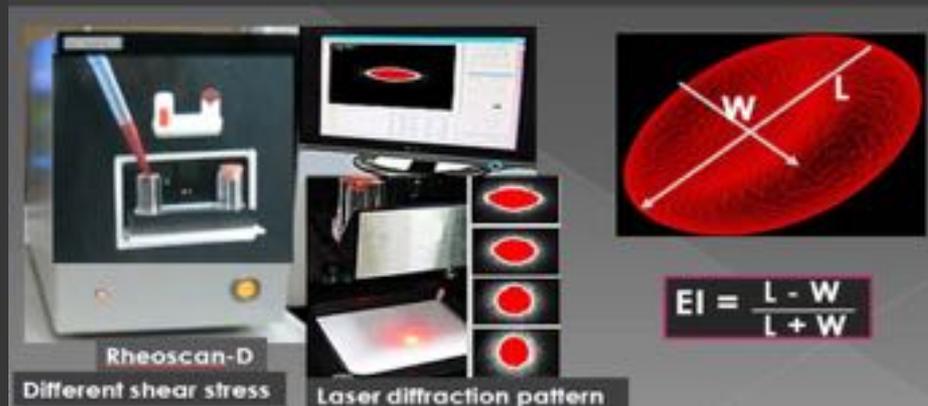


- a microchip-based cell aggregometer (Rheoscan-A; Rheo Meditech, Korea)
- from the sylectogram : a curve of the relationship between laser backscatter intensity and time.
- **Aggregation indices (AIs, %)** : the ratio of the area under the sylectogram to the total area over a 10-second period
- **Aggregation half time ($T_{1/2}$, s)** : the time to half-maximal aggregation



Analytical procedure

○ RBC deformability



- a microfluidic ektacytometer (Rheoscan-D; Rheo Meditech, Seoul, Korea)
- Erythrocyte images recorded during flow of the suspension in the slit
- Elliptic form of RBCs by the diffraction pattern **at the shear stress(0.09–20 Pa)**
- **Elongation indices (EIs)** : $(L-W) / (L+W)$, major and minor ellipse axes at shear stress values of **0.3, 0.5, 1, 3, 7, 10, and 20 Pascals(Pa)**
: the impairment of erythrocyte membrane resilience-elastic properties
- **Elmax** : a theoretical maximum EI value at infinite stress



Analytical procedure

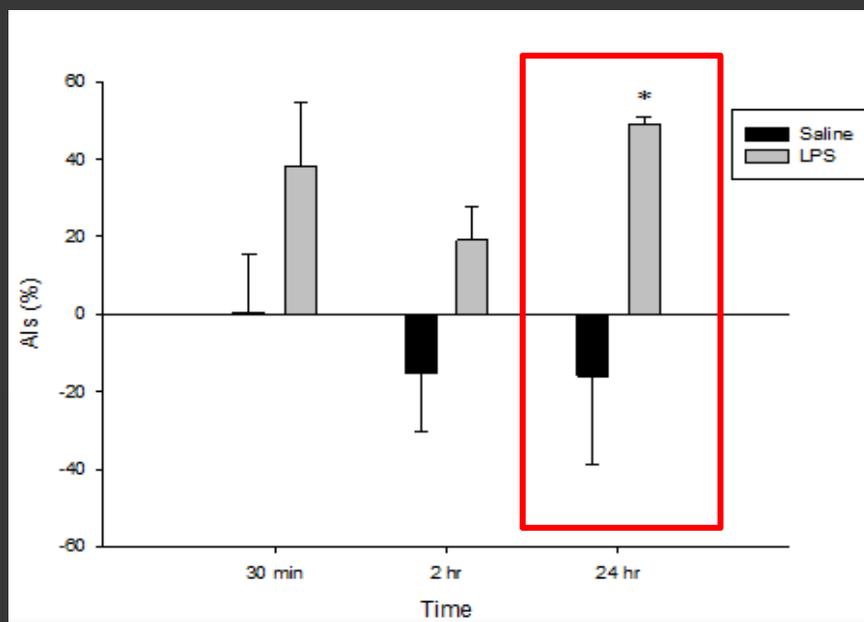
- ◉ Serum **pro-inflammatory cytokines** (interleukin [IL]-6 and IL-12) levels
 - using enzyme linked immunosorbent assay[ELISA] kits (R&D Systems, Minneapolis, MN, USA)
 - Serum collection : Blood centrifuge at $4,000 \times g$ and $4^{\circ} C$ for 10 min
 - IL-6 and IL-12 detection ranges : 7.80–500 pg/mL, respectively



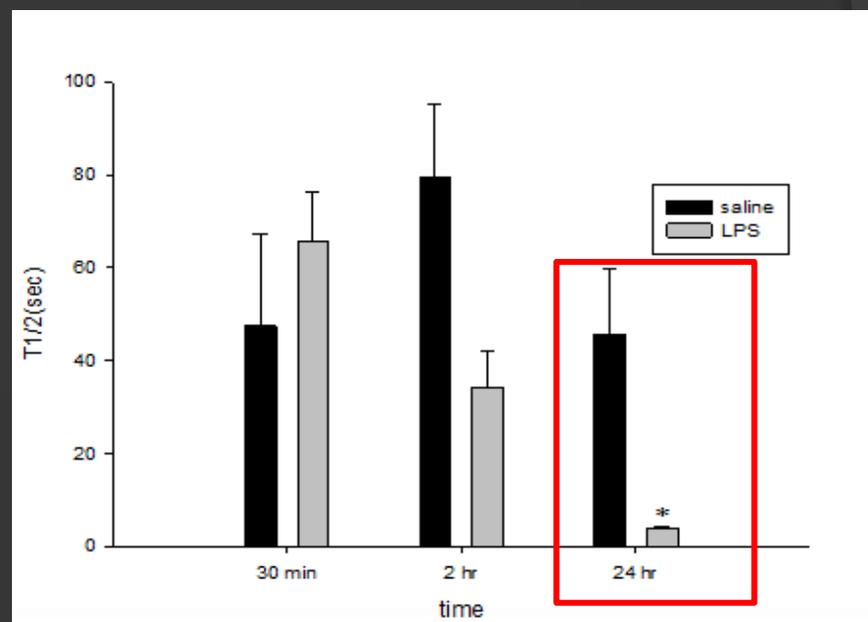
Results

RBC aggregation index (AI in %) and aggregation half-time ($t_{1/2}$ in s) at LPS versus saline injected group

Als(%)



T1/2(s)

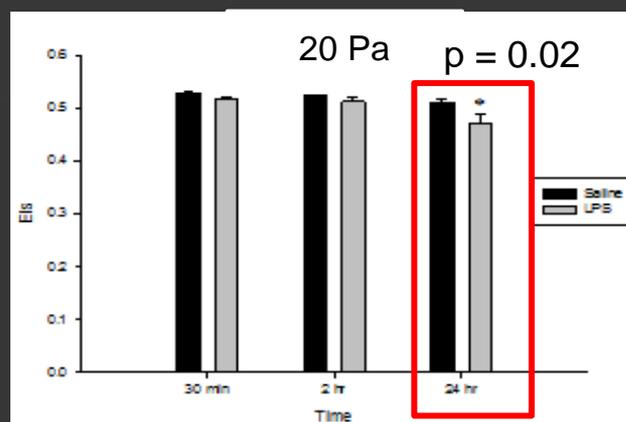
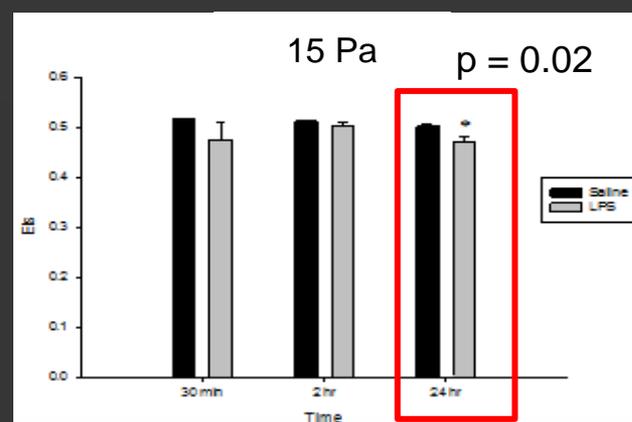
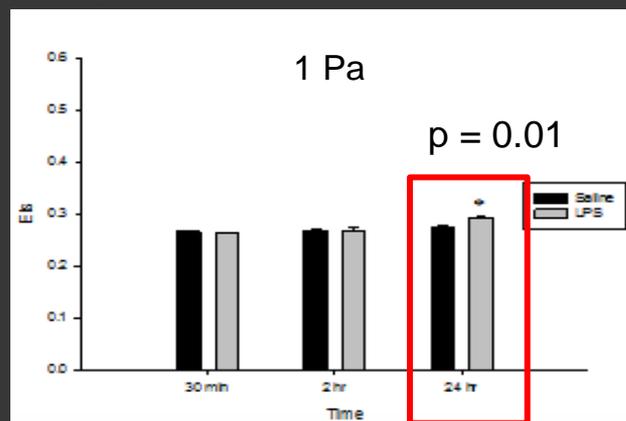
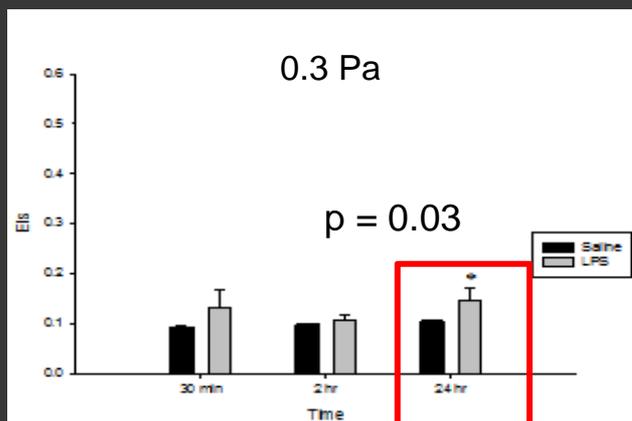


- $p = 0.01$, respectively
- * : $p < 0.05$, LPS vs. Saline



Results

RBC deformability (elongation indices, EIs) at each shear stress values at LPS versus saline injected group



* $p < 0.05$ LPS vs. saline

Elmax : no difference between the two groups



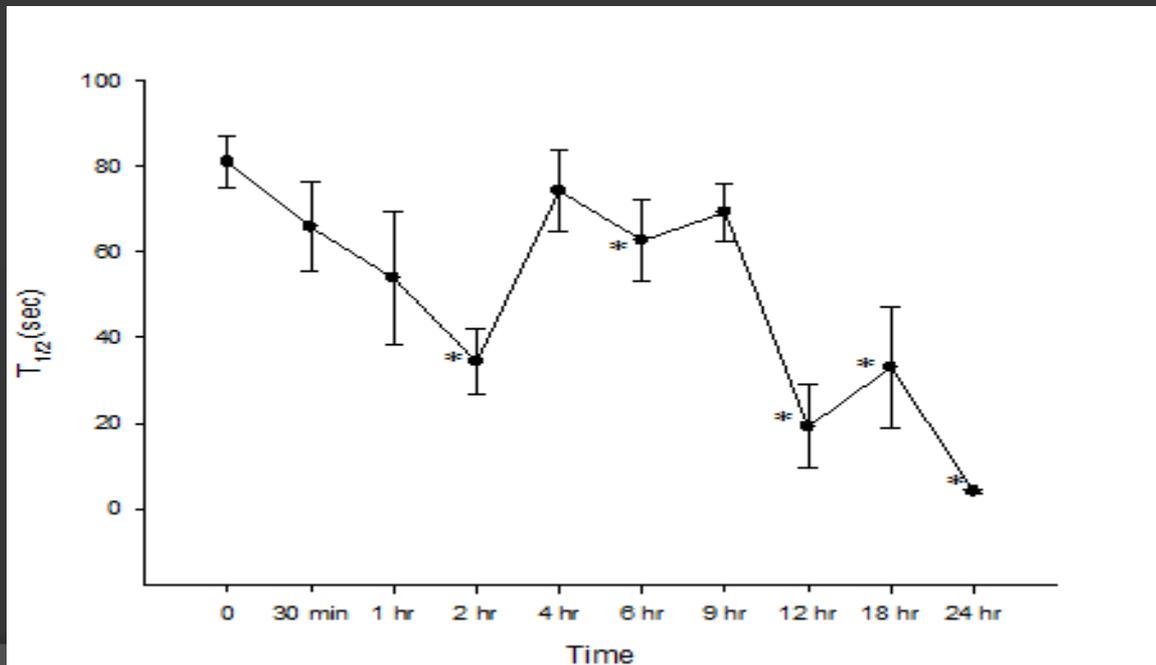
Results

< Sub-experimental group >

- 0, 30min, 1, 2, 4, 6, 9, 12, 18 and 24 hours after the LPS injection

RBC aggregation index (AIs) : no significant difference during the period

RBC aggregation half time($T_{1/2}$) : shorter at 2,6,12,18, 24hr than at baseline



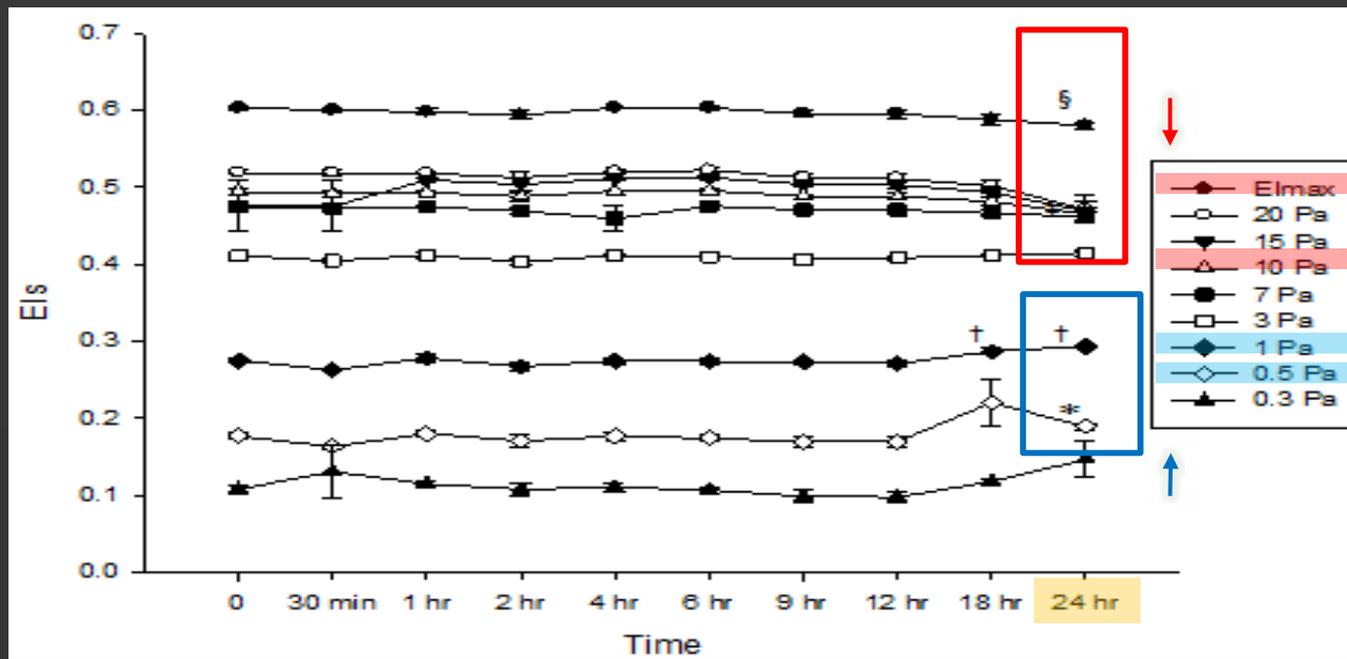
* $p < 0.05$ vs. baseline (0 hr)



Results

< Sub-experimental group >

RBC deformability (elongation indices, EIs) at each shear stress values and EImax in the LPS injected mice.



* $p < 0.05$ vs. baseline (0 hr) at 0.5 Pa ($p = 0.0038$, increase)

† $p < 0.05$ vs. baseline (0 hr) at 1 Pa ($p = 0.005$, increase)

‡ $p < 0.05$ vs. baseline (0 hr) at 10 Pa ($p = 0.02$, decrease)

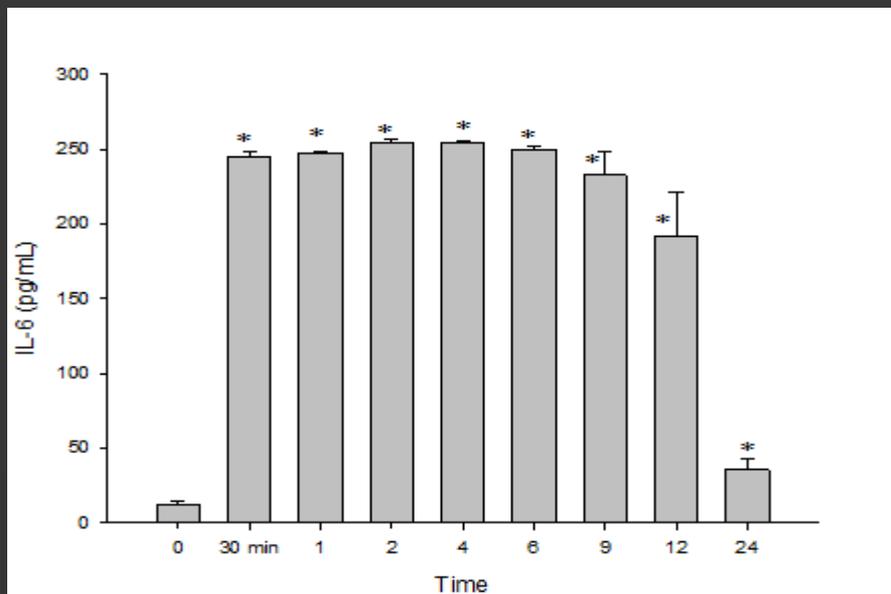
§ $p < 0.05$ vs. baseline (0 hr) at infinite stress ($p = 0.01$, decrease)



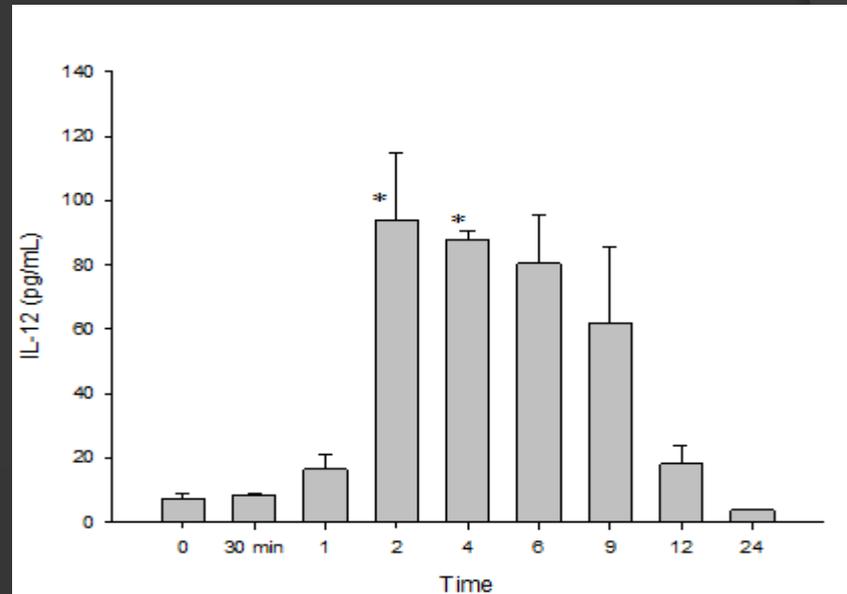
Results

Serum IL-6 and IL-12 concentrations in LPS injected mice

IL-6



IL-12



* $p < 0.05$ vs. baseline (0 hr)



Conclusions

- RBC aggregation and deformability changed in the LPS induced sepsis animal model
- Altered RBC deformability : detectable 24hr after the LPS injection in mice.
- Shear stress : should be considered to detect changes in RBC deformability in LPS-induced septic mice.
 - dual pattern : more deformable at low shear stress and stiffer at high shear stress during sepsis.
- $T_{1/2}$: a sensitive marker for detecting changes in RBC aggregation.

Thank you for your listening