The Effects of Bilirubin of Immune-inflammatory Response in Septic Neutrophil and Mortality in septic mouse

Chung Sung-Su M.D.¹ Song Jia M.D.¹ Kwak Sang-hyun M.D.¹

Department of Anesthesiology and Pain Medicine, Chonnam National University Hospital, Gwangju, South Korea

ABSTRACT

Background: Bilirubin is yellow compound that attend the heme catabolism pathway, was excreted in bile and urine, high levels may induce certain diseases. However, Bilirubin have potent antioxidant, important role anti-inflammatory in the animal sepsis model, protect the body against endotoxin-induced lung inflammatory, down-regulate expression of adhesion molecules, inhibit infiltration of inflammatory cells (specific is neutrophil in inflammatory response). Thus, Bilirubin is one of candidate for improving protect the body against inflammation-induced sepsis. The purpose of this study was to investigate the effect of Bilirubin on LPS-induced human neutrophil activation, and improve mortality on animal multiple organ failure (MOF) sepsis model.

Material and method: Human neutrophils were incubated with varying concentrations of Bilirubin (10μM, 50 μM, 100 μM) with or without LPS. Apoptosis is evaluated by FACS (Anexin V/ PI) after 24hr to determine delay apoptosis of Neutrophil in cycle cell. Next, we measured protein levels for tumor necrosis factor-alpha (TNF-a), interleukin (IL)-6 and IL-8 after 4 h incubation period. To clarify the intracellular signaling pathway, we measured the levels of phosphorylation of p38, NFkb. Then, we continue to investigate mortality rate on animal MOF sepsis model.

Results and conclusions: Bilirubin decreased activation of intracellular signaling pathways, including p38 and NFkb, and down-regulation expression of pro-inflammatory cytokines, including TNF-a, IL-6, IL1. Beside, increase significant neutrophil apoptosis in group treat than treat LPS. Furthermore, Bilirubin improve inflammatory on animal model, increase survival rate on model treat with Bilirubin. Bilirubin attenuate LPS-induced human neutrophils responses and support to improve mortality rate on animal model. We suggest that it effects could play an important role in modulating-mediated inflammatory responses.

Key words: Neutrophil, Lipopolysaccharide, cecal ligation and puncture

RESULTS (continue)

Figure 2. The change expressions of inflammatory protein level TNF-α (A), IL-1β (B), IL6 (C) When human neutrophil were cultured with and without LPS or Bilirubin (10μM, 50 μM, 100 μM) for 4hr. Protein results were obtained from ELISA (*p<0.05 versus control. †p<0.05 versus LPS alone.)

Figure 3. Bilirubin attenuates p38 kinase and NFkb protein expression induced by LPS on human neutrophil. Neutrophils were incubated for 30min with without or LPS or Bilirubin (10μM, 50 μM, 100 μM).

Figure 4. Survival rates of the C57BL/6 mice after suffered surgery “two hit” to make animal multiple organ failure model. Mice injected with LPS (5mg/kg body weight, *i.p.*) or saline (Control) were challenged with CLP at 24 h post-LPS (LPS-24h), then survival was observed for 15 days from CLP injury (10 mice/group, Kaplan-Meier plots followed by log-rank test)

CONCLUSION

In our study suggest that Bilirubin attenuate LPS- induced neutrophil activation, decrease release inflammatory cytokine TNF-α, IL-6, IL-1β and down-regulation expression intracellular signaling pathways, including p38 and NFkb. Furthermore, apoptosis rates of neutrophils increase significantly in group treat with bilirubin when compare with group treat LPS, this is explained because inflammatory cytokine is factor prolong life and delay neutrophil undergoing apoptosis in cell cycle, attenuate expression level cytokine is reason lead to stimulate neutrophil go apoptosis.

In this study, we first step found Bilirubin have potent that improve mortality rate on mice early MOF model. This is new strategy to treat and recover sepsis patient in the future.

RESULTS

Figure 1. Apoptosis rates of Human Neutrophil when they were challenged with and without LPS or Bilirubin (10μM, 50 μM, 100 μM) after 24hr cultured. The results are expressed as mean SD from three independent experiments (*p<0.05 versus control. †p<0.05 versus LPS alone.)