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Brain-derived neurotrophic factor (BDNF) is a prognostic biomarker and therapeutic target through AMP-activated protein kinase (AMPK) signal pathway for sepsis

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Introduction

- **Brain-derived neurotrophic factors (BDNF)** are derived from the brain, but they are known to act as cytokines in various fields, including metabolic control and support for hematopoietic action as well as the central nervous system.
- A recent study showed **that the level of this BDNF were independently associated with mortality in severe patients.**
- **AMP-active protein kinase (AMPK)** is known to have anti-inflammatory effects by inhibiting the secretion of pro-inflammatory cytokine by regulating **NF-κB signals**.
- We conducted studies with the aim of confirming **the possibility of BDNF as a prognostic marker for sepsis, reducing inflammation-related cytokine by acting on AMPK**.

Material & Methods

- **Experiment-1**

For a study on sepsis patients, **BDNF was measured and compared with 168 sepsis patients who visited the emergency room** of our hospital from January 2017 to December 2018 and **48 healthy volunteers as controls**.

- **Experiment-2**

Experiments were conducted to **compare AMPK activation before and after BDNF treatment** with changes in the amount of **pro-inflammatory cytokine related to the expression of NF-κB signal induced by LPS**, a representative bacterial toxin.

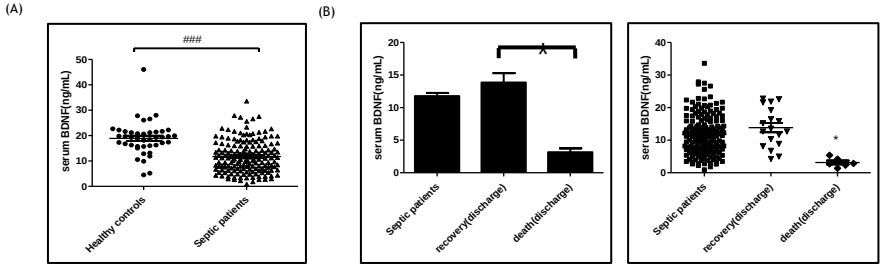
We conducted this experiment under
***in vitro* environment** using RAW264.7 cells, monocyte/macrophage-like cells of mice,
***ex vivo* environment** using peritoneal macrophage extracted from mice,
and ***in vivo* environment** using animal sepsis modeling(CLP), respectively.

Results - 1

Human serum BDNF ELISA

(A) **The mean value of the control group was 18.91ng/mL, and the mean value of the sepsis patients was 11.78ng/mL, showing significant difference**. **### *p*<0.001** versus Healthy control group.

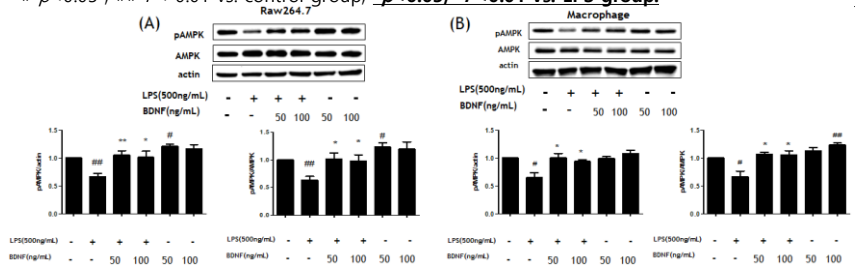
(B) Among 168 sepsis patients, BNDF level of 24 patients was measured at discharge. The mean value of 18 patients who **recovered and discharged was 13.86 ng/mL and the mean value of 6 patients who died was 3.16 ng/mL**, also showing significant difference. ***p< 0.05 versus recovery group**.



Results - 2

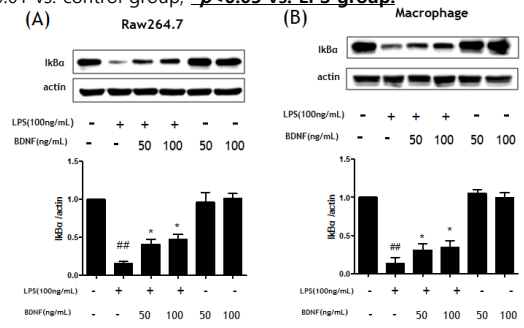
BDNF on AMPK pathway

(A)(B) **LPS stimulation suppress pAMPK expression, and BDNF treatment restore pAMPK expression** in both LPS stimulated RAW264.7 cells and Mouse peritoneal macrophage cells.
# *p*<0.05 , ## *P*< 0.01 vs. control group; ***p*<0.05, **P*<0.01 vs. LPS group.



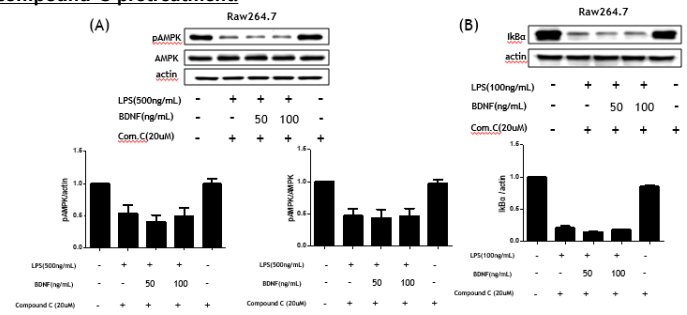
BDNF on NF-κB pathway

(A)(B) **LPS stimulation suppress expression of IkBα, and BDNF treatment restore IkBα expression in both LPS stimulated** RAW264.7 cells and Mouse peritoneal macrophage cells.
# *p*<0.05,##*P*<0.01 vs. control group; ***p*<0.05 vs. LPS group.



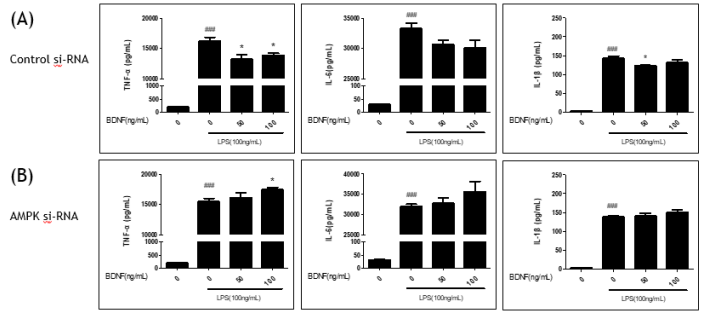
AMPK inhibition of compound C (AMPK inhibitor)

(A) **BDNF treatment do not restore pAMPK expression** in LPS stimulated RAW264.7 cells **after compound C pretreatment**.
(B) **BDNF treatment do not restore IkBα expression** in LPS stimulated RAW264.7 cells **after compound C pretreatment.**



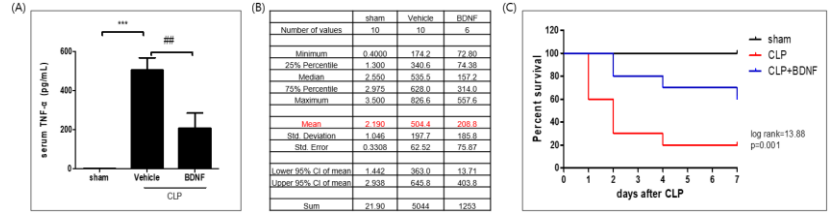
Cytokine analysis by ELISA-AMPK knockdown

(A)(B) **The effect of suppressing LPS-induced pro-inflammatory cytokine expression of BDNF was not observed in siRNA for AMPK** transfected RAW264.7 cells.
###*p*<0.001 vs. control group; ***p*<0.05 vs. LPS group.



Comparison of pro-inflammatory cytokine level & survival rate of murine sepsis model

(A)(B) TNF-α, a representative cytokine, was rarely produced in the Sham group that only opened and closed the abdominal cavity, but increased in the CLP groups that caused sepsis. In addition, **TNF-α is significantly reduced in the group treated with BDNF among the CLP group compared to the group treated without BDNF(Vehicle); ; ##*p*<0.01 vs. CLP**



	sham	Vehicle	BDNF
Number of values	10	10	6
Minimum	0.4000	174.2	72.80
25% Percentile	1.300	340.6	74.38
Median	2.500	536.5	157.2
75% Percentile	2.975	626.0	314.0
Maximum	3.500	826.6	557.6
Mean	2.190	504.4	208.8
Std. Deviation	1.046	197.7	185.8
Std. Error	0.3308	62.52	75.87
Lower 95% CI of mean	1.442	363.0	13.71
Upper 95% CI of mean	2.938	645.8	403.8
Sum	21.90	5044	1253

(C) **When follow up to the 7th day, the BDNF group showed a significant increase in the survival rate than the Vehicle group.; log rank=13.88, *p*=0.001**

Conclusion

- **BDNF increases in sepsis and tends to be lower in people with poor prognosis among sepsis patients at discharge**.
- BDNF passes through the **AMPK pathway** and can reduce pro-inflammatory cytokine in sepsis **by regulating NF-κB signal**.
- **In the animal experimental sepsis model**, it was confirmed that the group treated **with BDNF had a higher short-term follow-up survival rate.**
- Our findings suggest that **BDNF can be applied to prognostic factors and treatment target targets in sepsis.**