LSIM Safety Institute

Drug evaluation of LPS-induced acute lung injury (ALI) mice models (2nd Report)

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Objective

In the 94th Annual Meeting of the Japanese Pharmacological Society, we reported two kinds of pulmonary inflammatory models using lipopolysaccharide (LPS): a LPS inhalation model and an α-GalCer-LPS instillation model. In the previous report, we concluded that these LPS-induced ALI models could be useful to evaluate therapeutic efficacy of drugs used for treatment of pneumonia.

In the present study, we carried out further research on the LPS instillation model with a pretreatment of α-garactosyl ceramide (α-GalCer).

We also examined effects the effect of exosome from the human adipose cell and anti-IL-6R antibody. We evaluated inflammatory cell infiltration in the BALF (Broncho Alveolar Lavage Fluid), BALF supernatant cytokine level and SpO_2.

Summary in Japanese

急性肺損傷（acute lung injury：ALI），急性呼吸促迫（窮迫）症候群（acute respiratory distress syndrome：ARDS）は，呼吸不全（肺機能不全）の総称で，新型コロナウイルス感染症の流行から注目を集めている．

急性肺障害の動物モデルは，LPSの吸入などにより作製し，短期間，かつ，簡便に実施できることから薬物のスクリーニングに用いられている．

本研究では，マウスにα-GalCerおよびLPSを処置することで作製した急性肺炎モデルに対する薬物の作用をSpO_2，BALF中炎症細胞浸潤ならびにBALF上清中サイトカイン濃度により検討した．

その結果，薬物それぞれに特徴的な効果が認められ，薬物による肺炎治療に対するツールとして有用であることが確認された．

Materials and Methods

Animal

BALB/cAnNCrlCrlj (Jackson Laboratory Japan, Inc.), ♂, 7 weeks old (at Day 0)

Reagents

LPS: Lipopolysaccharides, from Escherichia coli O111:B4, Sigma-Aldrich Inc.

α-GalCer (α-galactosyl-ceramide): Kyowa Hakko Kirin Co., Ltd.

Dexamethasone: Sigma-Aldrich Inc.

Exosome: Preadipocyte Exosomes (human), ZENbio

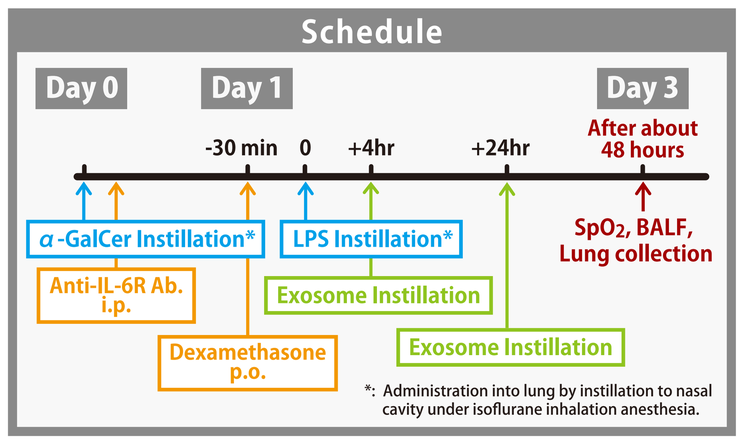
Anti-IL-6R antibody: InVivoMAb anti-mouse IL-6R, BioXcell

Measurement System

SpO_2: Mouse OxPLUS (primetech)

Cytokine: Bio-Plex Multiplex Immunoassay System (Bio-Rad Laboratories, Inc.)

Schedule



Discussion

Evaluation of the LPS-induced lung injury animal model was carried out by administrating various reagents. Reagents showed characteristic effects on this animal model. The results indicate that characteristic pathophysiological changes in the present model was identified and also suggested that this model is very useful for ALI study.

- **Dexamethasone might be useful for medical treatment of pneumonia at a high dose.**
- **To explore the possibility that the Exosome may have a therapeutic effect, it may be necessary to change the timing of administration, etc.**
- **Anti-IL-6R was partially effective (cytokine), it may be necessary to change the timing of administration, etc for further confirmation of effectiveness.**

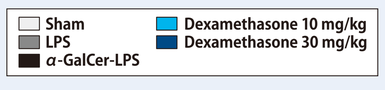
Results

Animal Model Confirmation

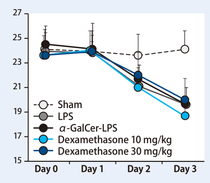
Group Configuration

Group	α-GalCer	LPS	Dose and Route	N
Sham	PBS	Saline	0 mg/kg, p.o.	4
LPS	PBS	5 μg		4
α-GalCer-LPS	1 μg	5 μg		4
Dexamethasone			10 mg/kg, p.o.	4
			30 mg/kg, p.o.	4

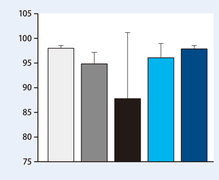
Results



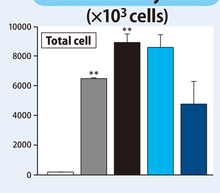
Body Wight (g)



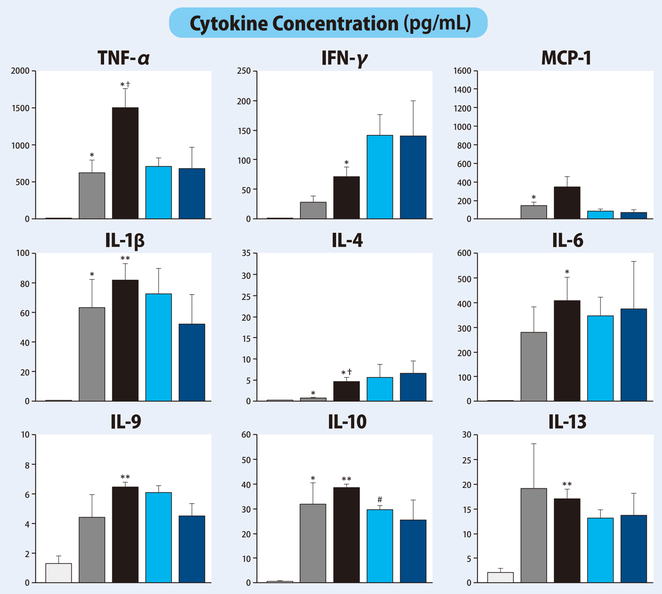
SpO_2 (%)



Inflammatory Cells (×10³ cells)

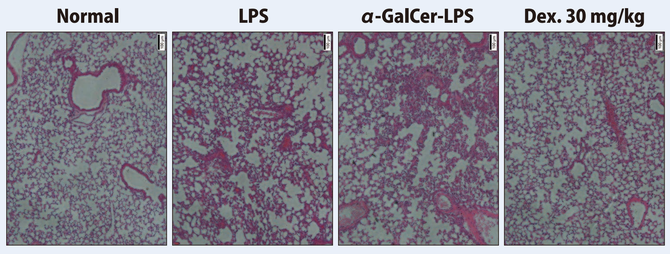


Cytokine Concentration (pg/mL)



*: p<0.05, **: p<0.01, significant difference compared to normal group (Student's or Aspin-Welch's *t*-test)
†: p<0.05, significant difference compared to LPS group (Student's or Aspin-Welch's *t*-test)
#: p<0.05, ##: p<0.01, significant difference compared to normal group (Dunnett's or Steel multiple comparison test)

Histopathological Study



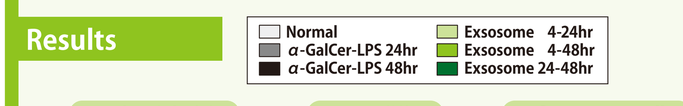
1. **Inflammatory cell infiltration and cytokine release were observed in the airways and lungs due to the induction of pulmonary inflammation.**
2. **Dexamethasone in excessive doses was effective to this reaction [cytokine level: IL-10, G-CSF (data not shown)].**

Exosome Study

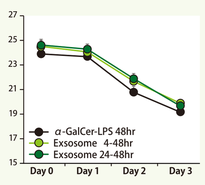
Group Configuration

Group	Adm. time (hr)	Sacrifice time (hr)	Dose and Route	N
Sham	4	48	0	3
α-GalCer-LPS 24hr	4	24		3
α-GalCer-LPS 48hr	4	48		3
Exsosome 4-24hr	4	24	1×10^{10} particles/body, Instillation	3
Exsosome 4-48hr	4	48		3
Exsosome 24-48hr	24	48		3

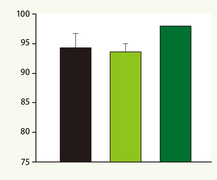
Results



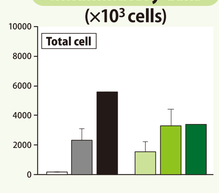
Body Wight (g)



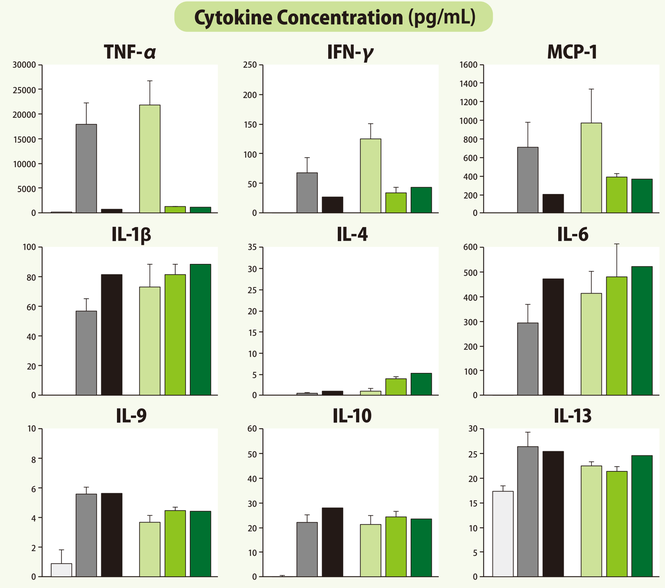
SpO_2 (%)



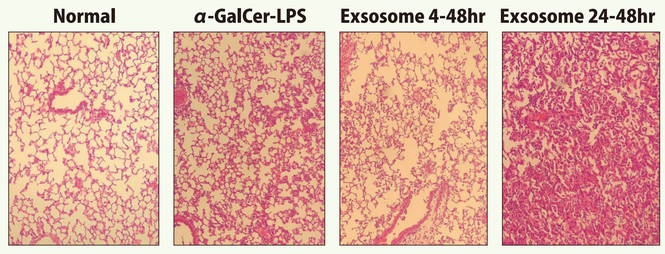
Inflammatory Cells (×10³ cells)



Cytokine Concentration (pg/mL)



Histopathological Study



1. **Exosome was suppressed the Inflammatory cell infiltration, but were not suppressed the inflammatory cytokine levels.**
2. **Depending on the timing of Exosome administration, lung inflammation was found to worsen.**

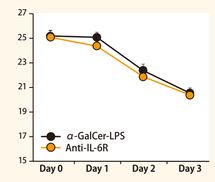
Anti-IL-6R Antibody Study

Group Configuration

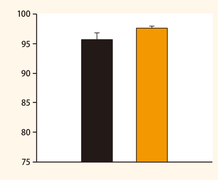
Group	Dose	Concentration	N
α-GalCer-LPS	0	0	5
Anti-IL-6R antibody	200 μg/body	1 mg/mL	5

Results

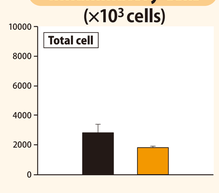
Body Wight (g)



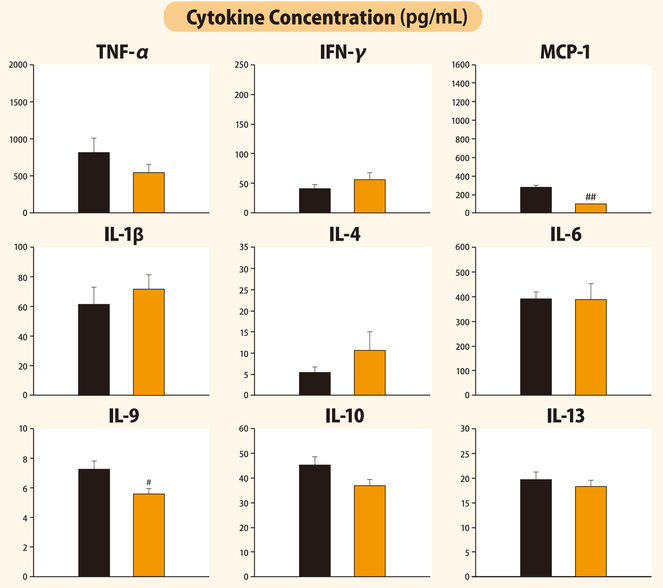
SpO_2 (%)



Inflammatory Cells (×10³ cells)



Cytokine Concentration (pg/mL)



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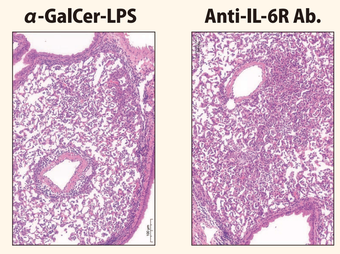
Histopathological Study



1. **Anti-IL-6R antibody was not suppressed the Inflammatory cell infiltration, but were partially suppressed the cytokine levels [MCP-1, IL-9, GM-CSF, IL-5, KC, and MIP-1α (data not shown)].**