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Evaluation and comparison of two kinds of LPS-induced acute lung injury (ARI) mice models

2種類のLPS誘発急性肺炎症モデルの評価と比較

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- Two kinds of LPS-induced acute lung injury mice models were confirmed that it able to evaluate.

① LPS inhalation model

Inflammatory cell infiltration in BALF was confirmed and dexamethasone (10 mg/kg) improved this infiltration.

② α-GalCer-LPS instillation model

A weight loss, the abnormality of the general state and lung inflammation after instillation of LPS were confirmed. Increase of the number of more remarkable cell infiltration in BALF was confirmed compared to LPS inhalation model. There is no significant difference, this infiltration was seemed to be improved by dexamethasone (30 mg/kg) under the study condition.

- As a result, these two models were different in a characteristic each, and were able to use for evaluating the drug.

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COI開示

☑ 発表内容に関連し、過去３年間、開示すべきCOI関係にある企業などはありません。

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責任発表者： 片山　誠一

Introduction

- Pneumonia is recognized as the serious condition of a patient by the expansion of the new coronavirus (COVID-19) infectious disease. In addition, in the new coronavirus infectious disease, the symptom of the acute pneumonia symptom to take a sudden turn is brought into problem in particular.
- LPS (lipopolysaccharide)–induced acute lung injury model is used for the study of acute pneumonia symptom most, and we prepared and compared two mouse acute lung injury model using the LPS.
- One is a model to let LPS expose to a whole body, and another one is instillation of LPS to nasal cavity under anesthesia with a pretreatment of α-garactosyl ceramid (αGalCer).
- These two animal model was evaluated by inflammation cell infiltration into BALF and so on.

Two LPS-induced acute lung injury model

Model 1

- LPS inhalation model

Reference: Natalia de Souza Xavier Costa et al. Early and late pulmonary effects of nebulized LPS in mice An acute lung injury model. PLoS ONE 12(9):e0185474.

Model 2

- α-GalCer-LPS instillation model

Reference: Hiroyasu Ito et al. Lethal endotoxic shock using α-galactosylceramide sensitization as a new experimental model of septic shock. Laboratory Investigation (2006) 86, 254–261

Tumurkhuu G. et al. The mechanism of development of acute lung injury in lethal endotoxic shock using α-galactosylceramide sensitization. British Society for Immunology, Clinical and Experimental Immunology, (2008) 152: 182–191

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Test System

○Animal

Mice: BALB/cAnNCrlCrlj (Charles River, Japan)
Sex: ♂
Age: 6 weeks old (at purchased)

○Reagent

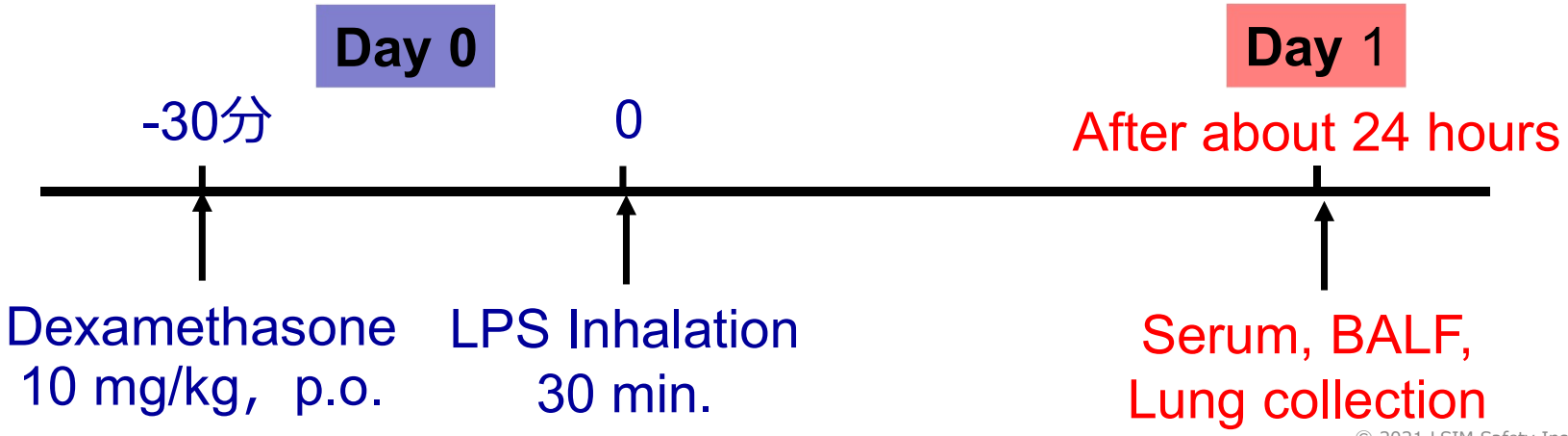
LPS: Lipopolysaccharides, from Escherichia coli O111:B4, Sigma-Aldrich Inc.
α-GalCer (α-galactosylceramide): Kirin, specific NKT activator
Dexamethasone: Sigma-Aldrich Inc.

Model 1 : LPS inhalation model

<Group configuration>

Group	LPS	Dose	N
Sham or Normal	-	-	3
LPS Inhalation	1 mg/mL, 30min	-	3～4
LPS Inhalation-Dexamethasone	1 mg/mL, 30min	10 mg/kg	3

<Schedule>



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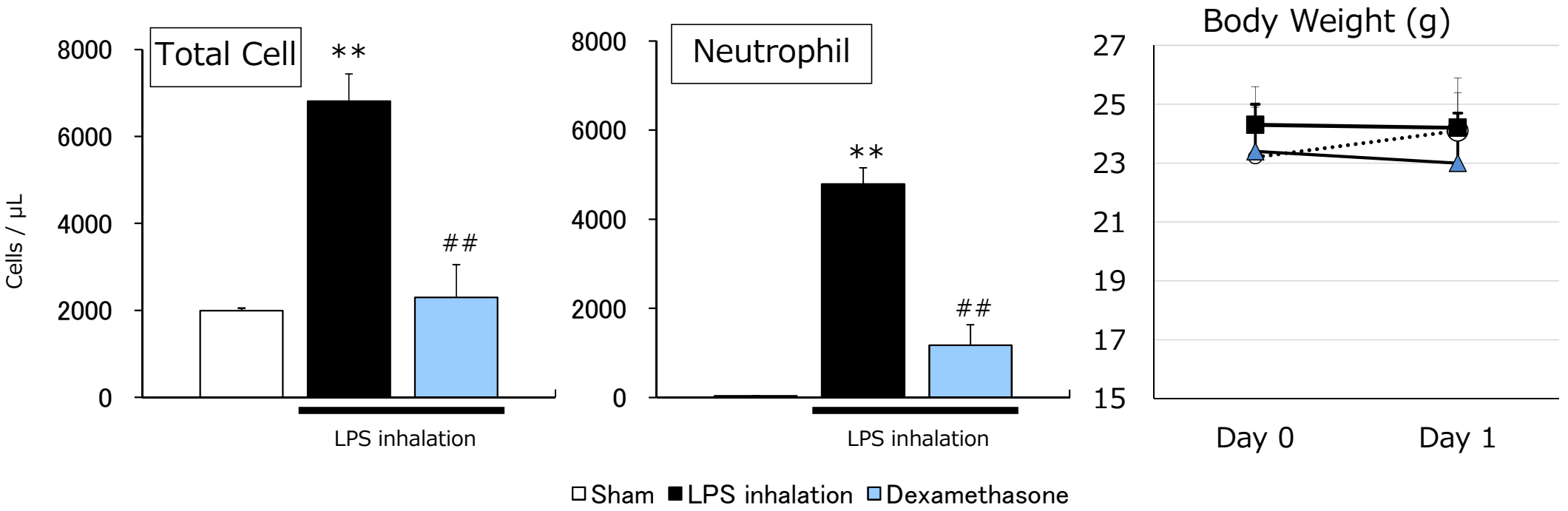
Model 1 : LPS inhalation model



**: p<0.01, Significant difference from Sham group (Student t-test)
##: p<0.01, Significant difference from LPS inhalation group (Student t-test)

Inflammatory cell infiltration in BALF, mainly neutrophil, induced by LPS inhalation was confirmed, and the change was improved by administration of dexamethasone.

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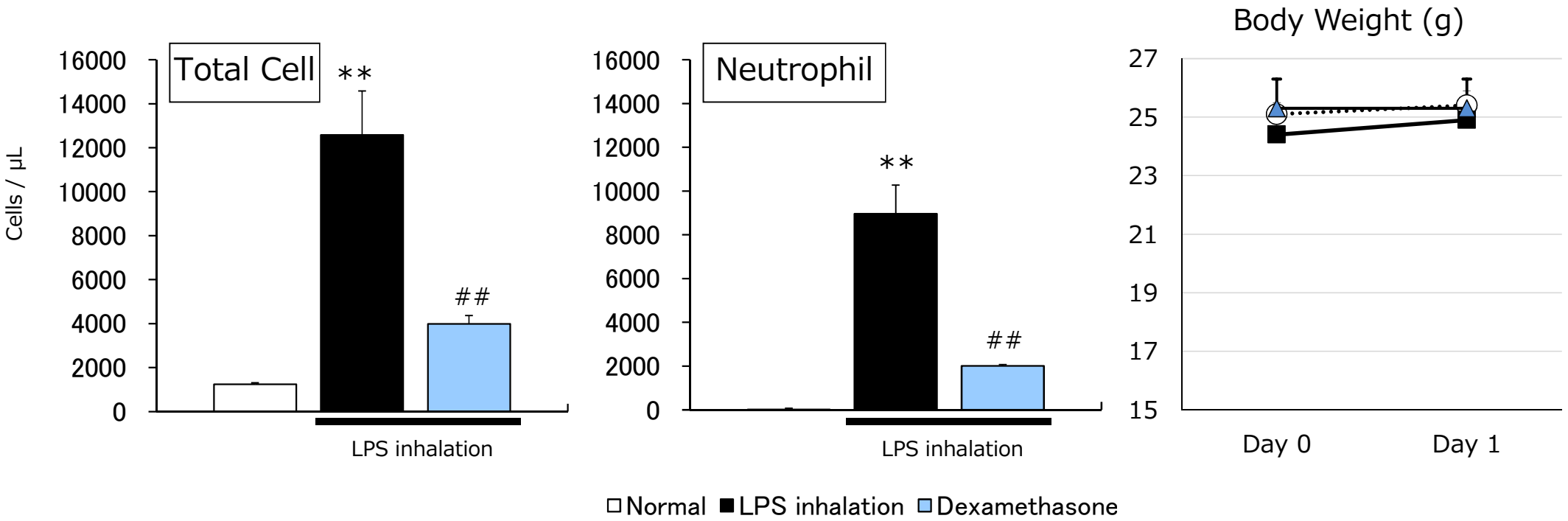
Model 1 : LPS inhalation model (reproducibility)



**: p<0.01, Significant difference from Normal group (Student t-test)
##: p<0.01, Significant difference from LPS inhalation group (Student t-test)

The reproducibility of lung injury induced by LPS Inhalation was confirmed, but the differences between experiments were existed.

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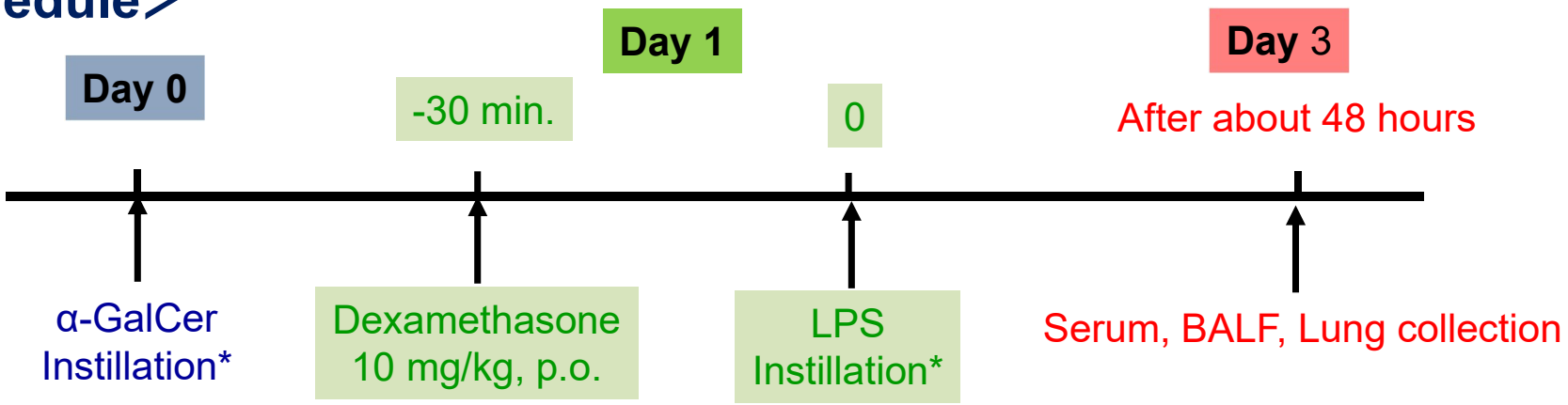
Model 2 : α-GalCer-LPS instillation model

<Group configuration>

Group	α-GalCer	LPS	Dose	N
Normal	-	-	-	3
α-GalCer-LPS	1 μg	5 μg	-	4
α-GalCer-LPS-Dexamethasone	1 μg	5 μg	10 mg/kg	3

<Schedule>



*: Administration into lung by instillation to nasal cavity under isoflurane inhalation anesthesia.

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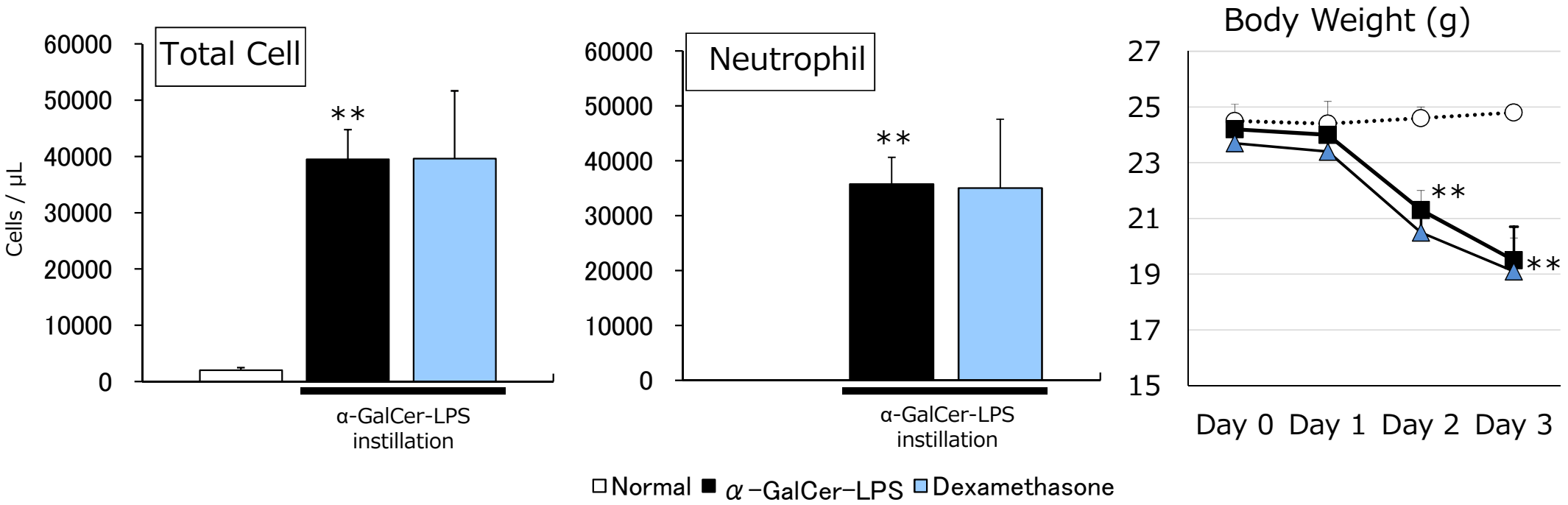
Model 2 : α-GalCer-LPS instillation model



**: p<0.01, Significant difference from Normal group (Student t-test)

Increase of the number of more remarkable cell infiltration in BALF was confirmed compared to LPS inhalation model. Furthermore, dexamethasone was not able to improve the increase. A weight loss, the abnormality of the general state and lung inflammation were confirmed.

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Model 2 : α-GalCer-LPS instillation model (reproducibility)

<Group configuration>

Group	α-GalCer	LPS	Dose (10 mL/kg)	N
Sham	PBS	Saline	Salline, p.o.	3
α-GalCer	1 μg			3
LPS	PBS	5 μg		3
Model (α-GalCer-LPS)	1 μg	5 μg		3
Dexamethasone			10 mg/kg, p.o.	3
			30 mg/kg, p.o.	3

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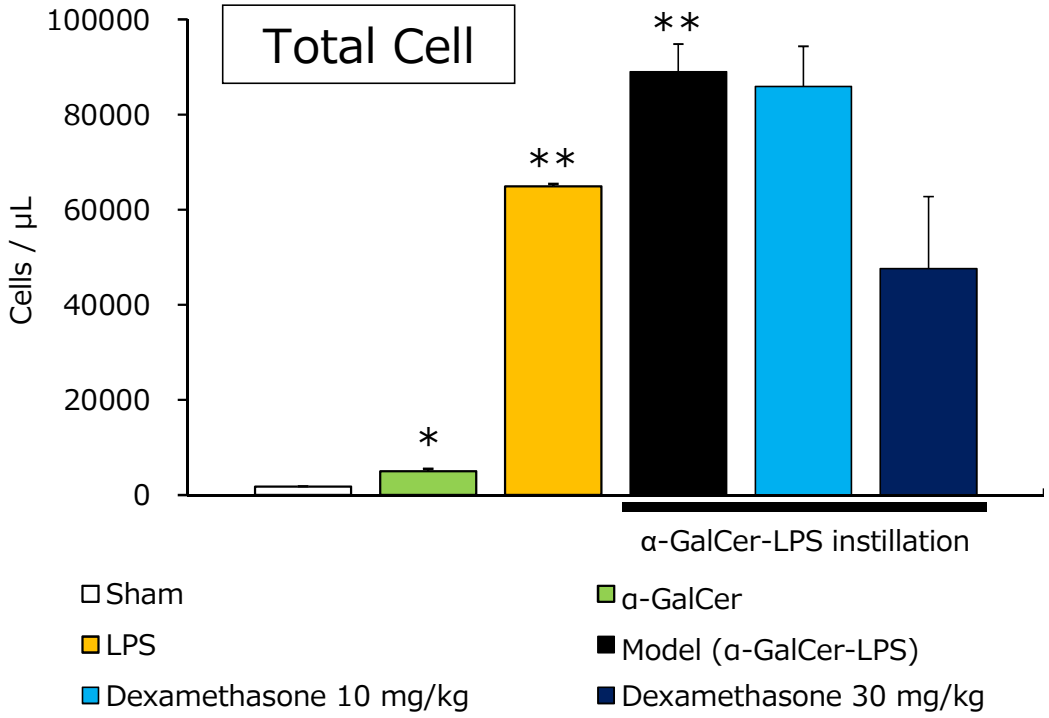
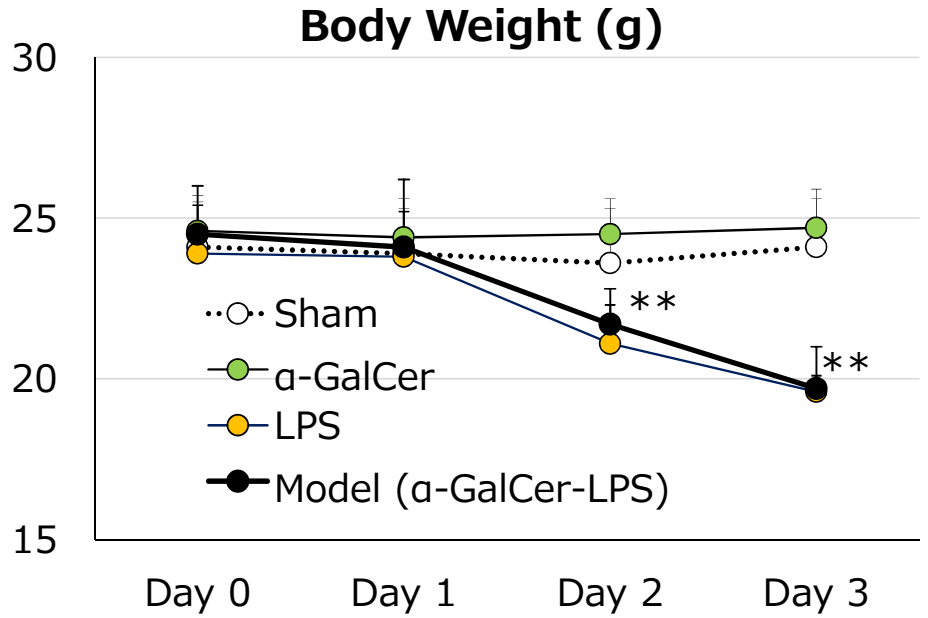
Model 2 : α-GalCer-LPS instillation model (reproducibility)





*: p<0.05, **: p<0.01, Significant difference from Sham group (Student t-test)

Inflammatory cell infiltration by only LPS instillation was confirmed sufficiently. However, by the instillation of α-GalCer, the infiltration was stronger compared with only LPS instillation. There is no significant difference, this infiltration was seemed to be improved by administration of dexamethasone (30 mg/kg).

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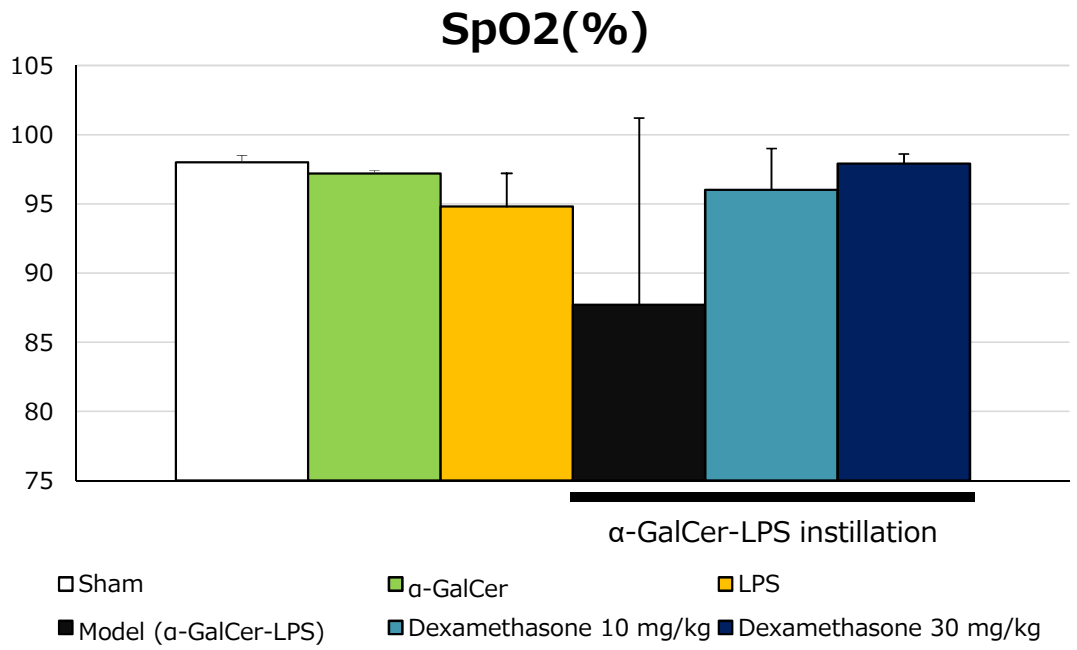
Model 2 : α-GalCer-LPS instillation model (oxygen saturation)



Reduction of SpO2 measured by pulse oximeter (Mouse OxPLUS, primetech) in Day 3 was confirmed in LPS and model group. There is no significant difference, this reduction of SpO2 was seemed to be improved by administration of dexamethasone.

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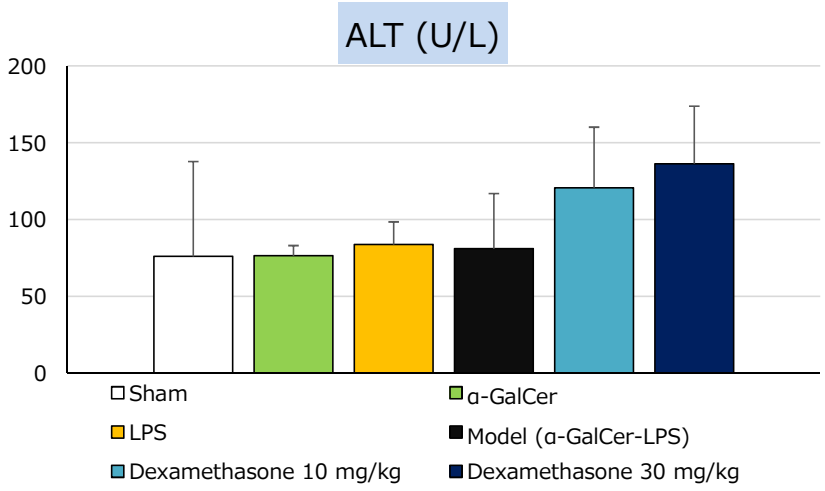
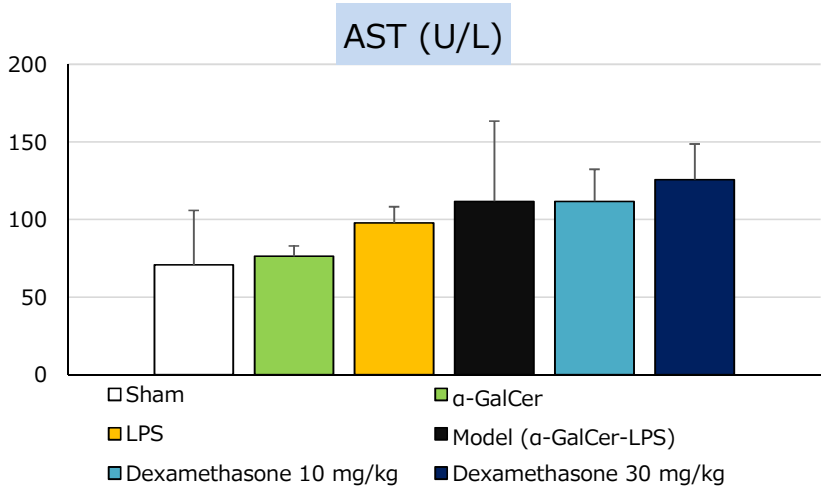
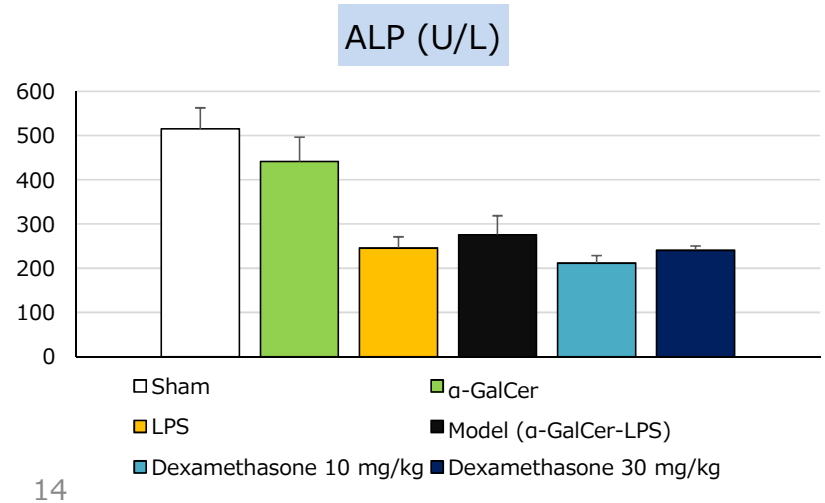
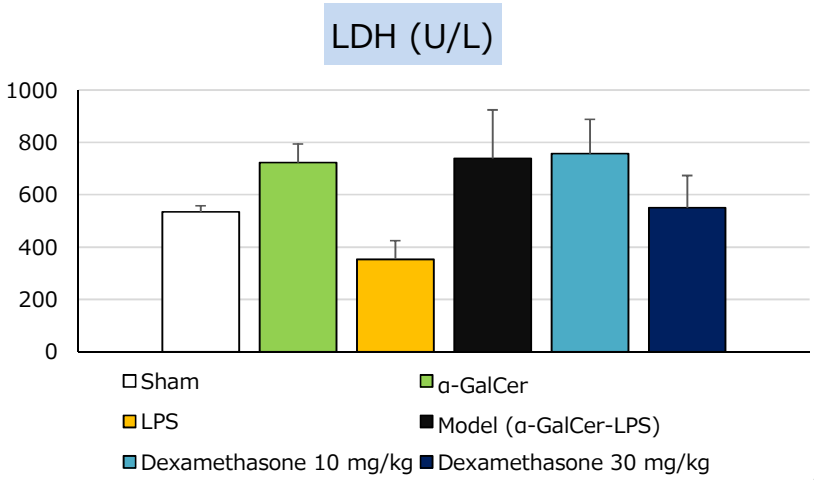
Model 2 : α-GalCer-LPS instillation model (biochemical examination)









No strong systemic inflammation was confirmed in this model as a result of measurement by the dry chemistry.

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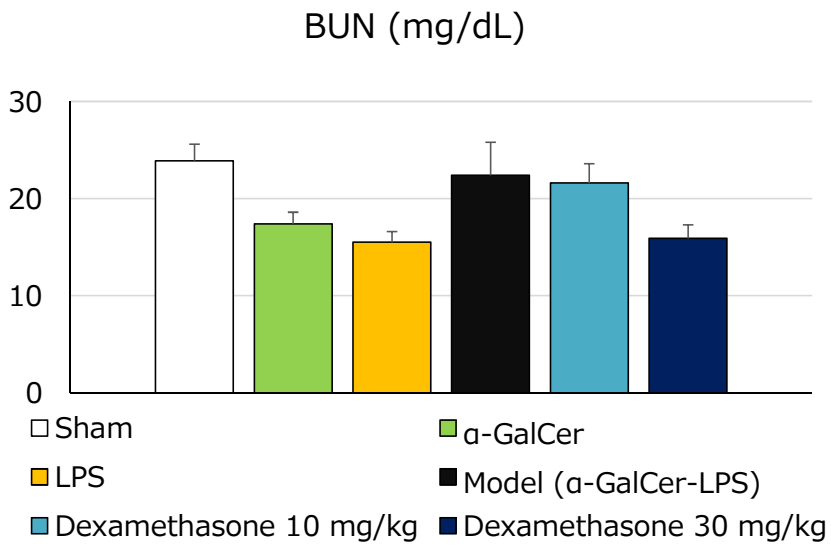
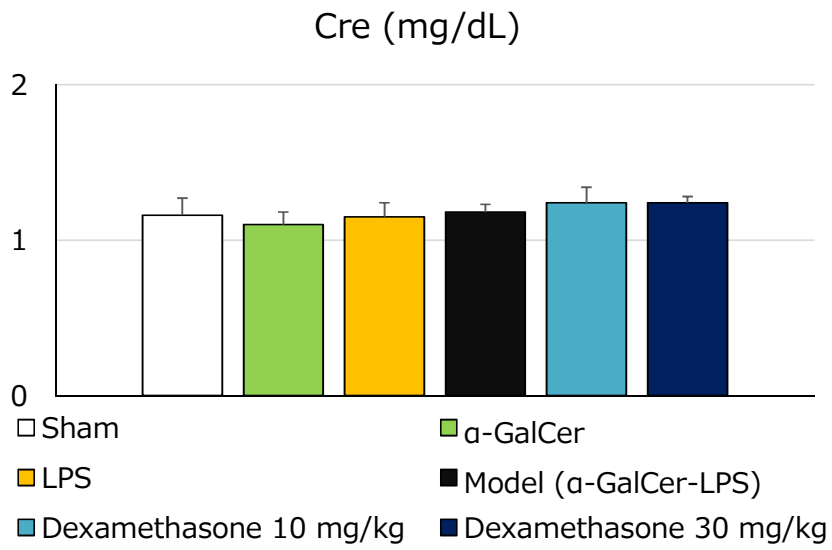
Model 2 : α-GalCer-LPS instillation model (biochemical examination)





No renal disorder was confirmed in this model as a result of measurement by the dry chemistry.

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Summary

・ Two kinds of LPS-induced acute lung injury mice models were confirmed that it able to evaluate.

① LPS inhalation model

Inflammatory cell infiltration in BALF was confirmed and this infiltration was improved by administration of dexamethasone (10 mg/kg).

② α-GalCer-LPS instillation model

A weight loss, the abnormality of the general state and lung inflammation after instillation of LPS were confirmed. Increase of the number of more remarkable cell infiltration in BALF was confirmed compared to LPS inhalation model. Furthermore, this infiltration was not improve by dexamethasone (10 mg/kg). And there is no significant difference, this infiltration was seemed to be improved by dexamethasone (30 mg/kg) under the study condition.

- Both models were confirmed that there was the difference between experiments.
- As a result, these two models were different in a characteristic each, and were able to use for evaluating the drug.
- About the cytokine storm, we are considering using BALF supernatant now.