Mediford Corporation

Evaluation of a polymicrobial sepsis mouse model induced by fecal suspension intraperitoneal injection as usefulness for clinical application.

○ Masateru Furuta, Souta Ushijima, Hajime Wada, Kousuke Morizumi, Seiichi Katayama, Naoyuki Hironaka, Katsuhide Nishi

Objective

Sepsis is caused by severe organ damage resulting from an uncontrolled immune response by infection. In treatment of sepsis in the early stage, antibiotics and other supportive therapy are used. Animal sepsis models have been made mainly by cecal ligation and puncture (CLP) or lipopolysaccharide (LPS) injection. However, these models are yet to be improved.

The objective of the present study was to induce the sepsis mice model by fecal suspension intraperitoneal injection (FSI model) and to evaluate validity of the model by administration of vancomycin and prednisolone. We inspected the survival rate, blood cytokine concentration, as well as histopathological analysis of intra-abdominal organs.

Summary in Japanese

敗血症 (Sepsis) は，生体への細菌感染により免疫反応が過剰に活性化する全身性の炎症反応である．最終的には臓器機能不全へ陥り，高い死亡率を示す．本報告において，我々は糞便懸濁液の腹腔内投与により，手術操作をせずに均質な多菌性敗血症を誘導できるモデル (FSIモデル) を作製した．さらに，本モデルに対するバンコマイシン及びプレドニゾロンの作用を生存率，血中サイトカイン濃度，腹腔内臓器の病理組織学的検査により検討した．

その結果，病態群では正常群と比較して，生存率の大幅な低下，血中における各種サイトカイン濃度の上昇が認められた．また，病理組織学的検査では，脾臓と回腸でマクロファージによる白血球の貪食，膵臓で菌体浸潤と壊死巣が認められた．敗血症様の所見が確認されたこの病態に対し，バンコマイシン投与は，生存率の有意な改善を示した．

本モデルは，血中サイトカイン濃度の上昇が認められたことから，高サイトカイン血症を生じるヒトの敗血症と類似しており，敗血症治療薬の薬効評価に有用であると考えられた．

Experiment 1： Confirmation and evaluation of mild FSI model

Animal

Male mouse, Slc:ICR, 7-weeks-old at model preparation

Methods

(1) The cecal contents collected from animals were suspended in PBS and filtered.
(2) Sepsis symptoms were induced by fecal suspension intraperitoneal injection at a dose of 1.6 g/kg.
(3) General condition was observed every 12 hours. Body weight was measured every day.
(4) Vancomycin or prednisolone was injected subcutaneously at arranged time.

Reagents

- PBS pH 7.4 (1×): Phosphate-Buffered Saline, Thermo Fisher Scientific.
- Vancomycin hydrochloride for injection: Okura Pharmaceutical Co., Ltd.
- Prednisolone sodium succinate for injection: Shionogi Pharma Co., Ltd.

Test group	Administration time after fecal suspension injection (hr)	Dose (mg/kg)	N
Vehicle Control	1, 6, 24*	0	5
Vancomycin	1, 6	10×2	5
Prednisolone	1, 24	0.1×2	5
Prednisolone	1, 24	1×2	5

＊ Vehicle was administrated to vehicle control group.

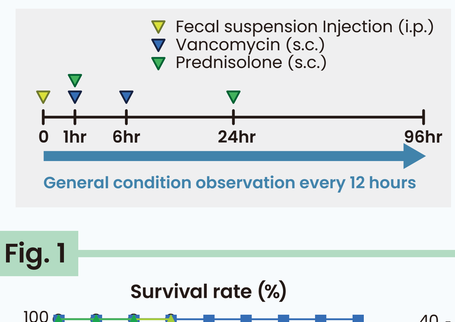
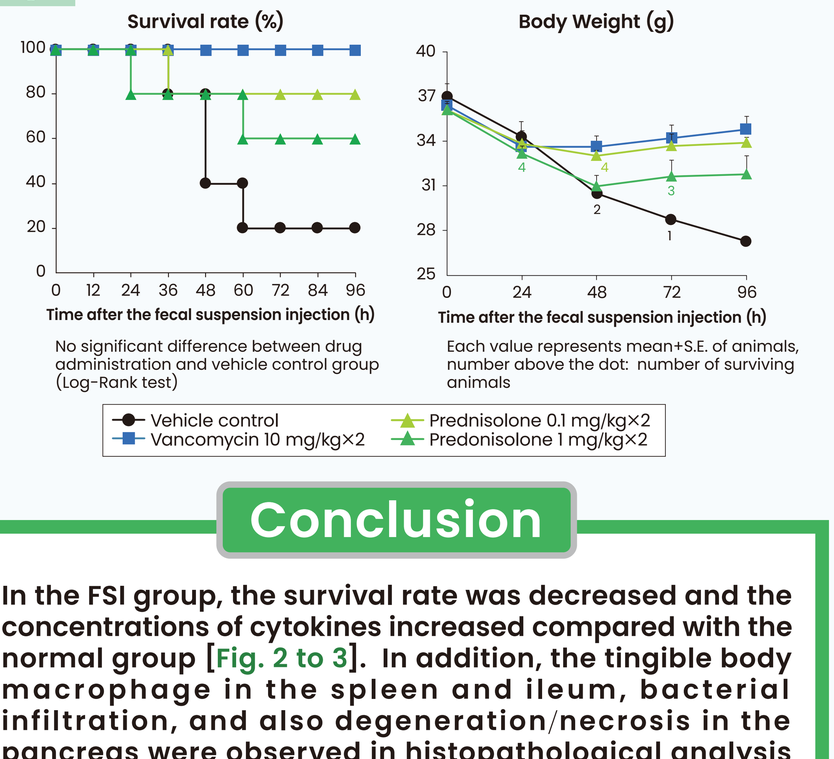


Fig. 1



No significant difference between drug administration and vehicle control group (Log-Rank test)

Each value represents mean+S.E. of animals, number above the dot: number of surviving animals

Experiment 2： The blood cytokine concentration and histopathological analysis in intense FSI model

Animal

Male mouse, Slc:ICR, 7-week-old at model preparation

Methods

(1) The model induction method was the same as **Experiment 1** (modification: 2.4 g/kg fecal suspension).
(2) Vancomycin or prednisolone was injected subcutaneously at arranged time.
(3) General condition was observed every 12 hours. Body weight was measured every day. [**Experiment 2-1**]
(4) Serum was collected 6, 24 hours after fecal suspension injection. [**Experiment 2-2**]
(5) Liver, kidneys, spleen, small intestine and pancreas were collected 24 hours after fecal suspension injection. [**Experiment 2-2**]

Reagents

The same as **Experiment 1**.

Measurement System

- Equipment: Bio-Plex Multiplex Immunoassay System (Bio-Rad Laboratories, Inc.)
- Kit: Bio-Plex Pro mouse cytokine G1 23-Plex panel (Targets: Eotaxin, G-CSF, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-17A, KC, MCP-1 (MCAF), MIP-1α, MIP-1β, RANTES, TNF-α)

Experiment 2-1

Test group	Administration time after fecal suspension injection (hr)	Dose (mg/kg)	N
Vehicle Control	2, 6, 24*	0	10
Vancomycin	2	10	10
Vancomycin	2, 6	10×2	10
Prednisolone	2, 24	20×2	10

＊ Vehicle was administered to vehicle control group.

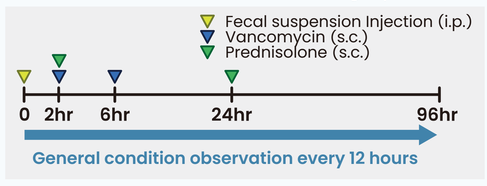
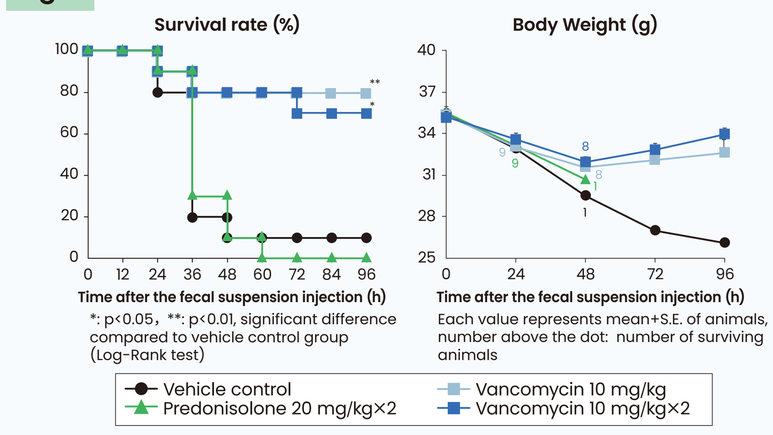


Fig. 2



*: p<0.05，**: p<0.01, significant difference compared to vehicle control group (Log-Rank test)

Each value represents mean+S.E. of animals, number above the dot: number of surviving animals

Experiment 2-2

Test group	Administration time after fecal suspension injection (hr)	Dose (mg/kg)	N
Normal	2*	0	10
Vehicle Control	2*	0	10
Vancomycin	2	10	10
Prednisolone	2	20	10
Satellite	-	-	10

＊ Vehicle was administered to normal and vehicle control group.

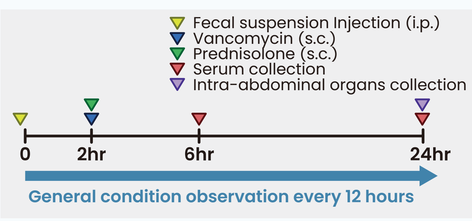
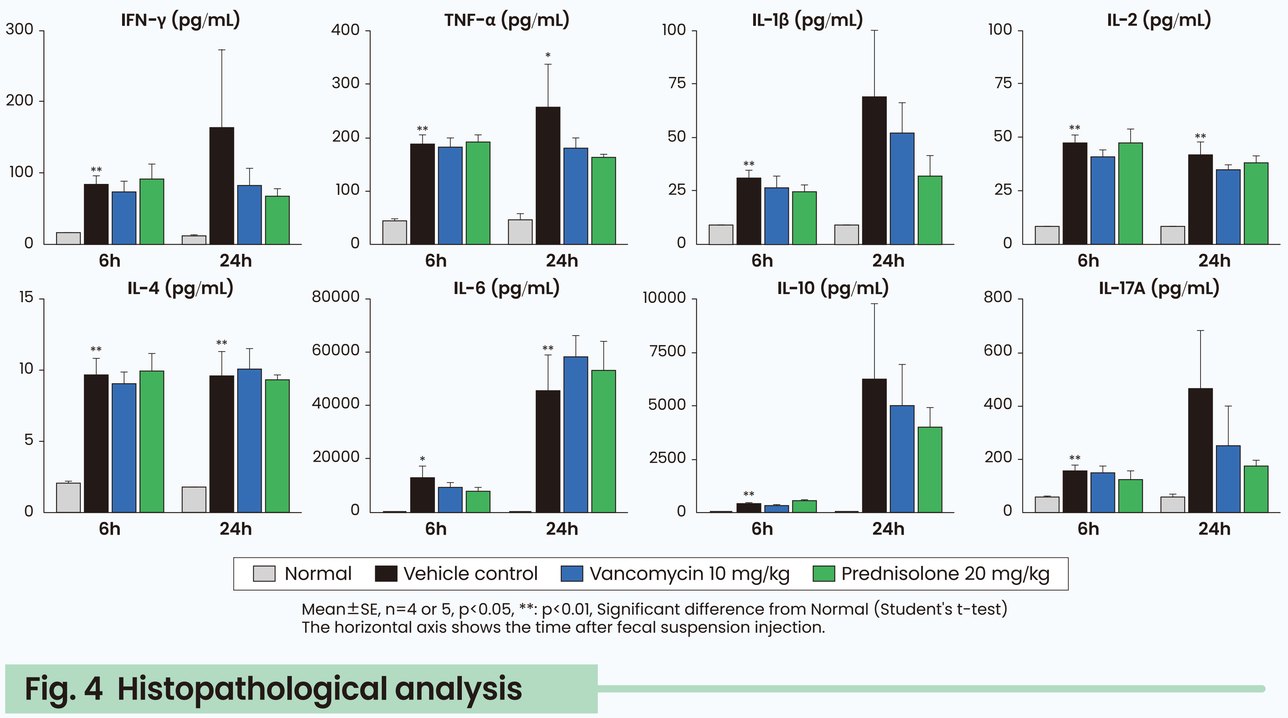
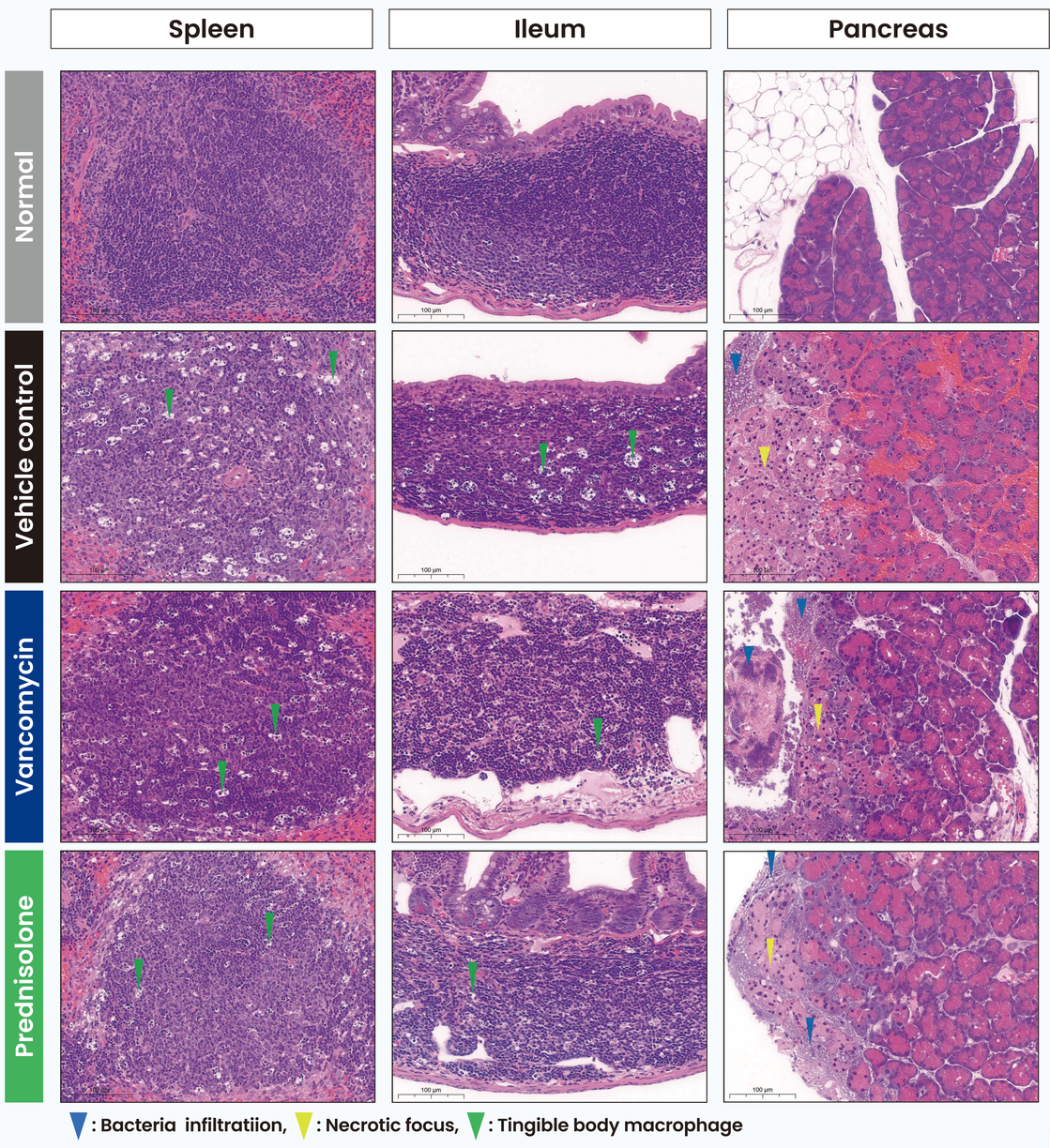


Fig. 3 Blood cytokine concentration



Mean±SE, n=4 or 5, p<0.05, **: p<0.01, Significant difference from Normal (Student's t-test)
The horizontal axis shows the time after fecal suspension injection.

Fig. 4 Histopathological analysis



Conclusion

In the FSI group, the survival rate was decreased and the concentrations of cytokines increased compared with the normal group [Fig. 2 to 3]. In addition, the tingible body macrophage in the spleen and ileum, bacterial infiltration, and also degeneration/necrosis in the pancreas were observed in histopathological analysis [Fig. 4]. Hypercytokinemia was caused by an increase in cytokines. Based on these results, the present model was considered to represent clinical signs of sepsis.

Vancomycin improved the survival rate in the FSI model [Fig. 2]. The improved survival rate by antibiotics was consistent with clinical results. However, vancomycin or prednisolone did not induce clear changes in cytokine concentrations or histopathological findings [Fig. 3 to 4]. This would be due to no anti-inflammatory effects of vancomycin, and the hypercytokinemia was too strong to suppress.

The FSI model was considered to be useful for evaluating the efficacy of drugs because the signs of this model were similar to the human sepsis.

mediford