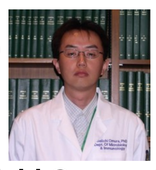
Efficacy of CMG Treatment and Microbiota Changes in a Mouse Model of Multiple Sclerosis

Seiichi Omura[1], Fumitaka Sato[1], Sundar Khadka[1], Yumina Nakamura[1], Aoshi Katsuki[1], Kazuto Nishio[1], Hideaki Kakeya[2], Ikuo Tsunoda[1]



PDF Poster

[1]Kindai University Faculty of Medicine,
[2]Graduate School of Pharmaceutical Sciences, Kyoto University

Seiichi Omura, PhD
omura.s@hotmail.com
http://tsunodalaboratory.web.fc2.com/

Abstract

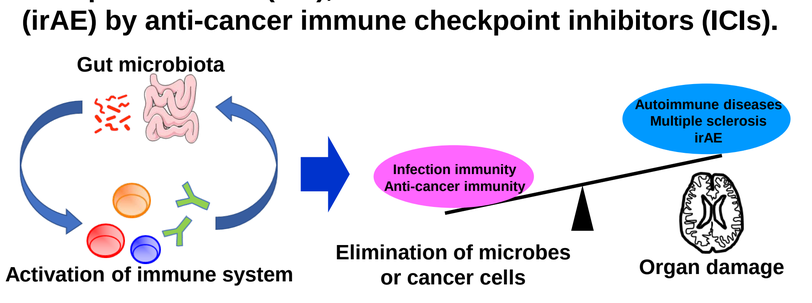
[Background and objective] Curcumin is a polyphenol compound, which is a component of *Curcuma longa*, and has been applied in clinical settings for several diseases, including cancer therapy. However, the oral administration of free-form curcumin has not provided the desired effect due to its metabolism during absorption from the intestine. Recently, we developed a prodrug type of curcumin (curcumin monoglucuronide, CMG), which is safe and can be injected intravenously. Intravenous injection of CMG showed an anticancer effect in the mouse model by achieving the blood concentration of free-form curcumin 1000-fold more than oral administration of curcumin. On the other hand, curcumin has been suggested its anti-inflammatory action and the effect on gut microbiota.

Multiple sclerosis (MS) is an inflammatory disease in the central nervous system (CNS), which causes myelin loss (demyelination) pathologically and various neurological signs, including motor paralysis. Experimental autoimmune encephalomyelitis (EAE) has been often used as an autoimmune model for MS. EAE is induced by the sensitization of animals with myelin components, including myelin oligodendrocyte glycoprotein (MOG). Recently, there are several reports suggesting the communication between the CNS and gut microbiota that may contribute to MS pathogenesis. We aimed to determine the efficacy of CMG treatment and the effect on microbiota in EAE.

[Results and discussion] We induced EAE in C57BL/6 mice by MOG sensitization. The mice were divided into four groups following MOG sensitization: the Control group was injected with PBS, the Induction group was injected with CMG for the first 5 days, the Latent group was injected with CMG from days 11 to 15, and the Whole group was injected daily with CMG (days 0 to 33). CMG-treated groups, particularly the Latent and Whole groups, showed decreased EAE scores clinically, compared with the Control group. On day 35, we harvested the CNS tissues and feces, ileum contents, and ileum mucosa. We found that CMG treatment decreased demyelination and inflammation with increased anti-inflammatory cytokines, IL-4 and IL-10. Using microbiome bioinformatics analyses, we demonstrated that CMG treatment affected to microbiota in feces and ileum content, but not ileum mucosa. Furthermore, principal component analysis (PCA) showed that principal component (PC) 1 values in the ileum content, but not feces, correlated with clinical signs and pathology scores. These results suggested that CMG treatment could alter microbiota in the ileum, contributing to suppression of EAE.

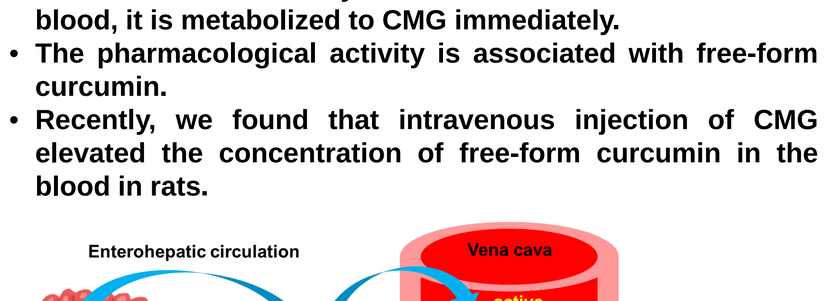
Introduction

- Communications between gut microbiota and immune system activate systemic immune responses that eliminate microbes and cancer.
- Uncontrolled excessive immune responses cause immune-mediated tissue damage, immunopathology.
- Immunopathology is seen in autoimmune diseases, multiple sclerosis (MS), and immune-related adverse event (irAE) by anti-cancer immune checkpoint inhibitors (ICIs).



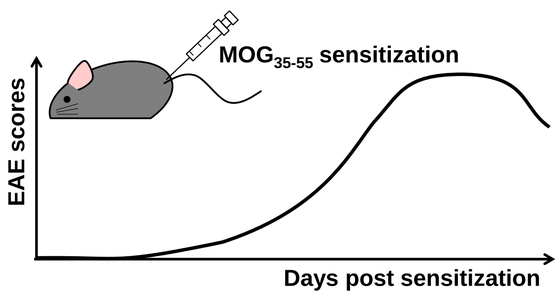
Curcumin monoglucuronide (CMG)

- Curcumin is a polyphenol compound and has various effects, including anti-tumor, anti-inflammation, and neuroprotection, as well as alteration of the microbiota.
- Free-form curcumin is highly lipophilic and is rarely absorbed into the body. Once curcumin is taken into the blood, it is metabolized to CMG immediately.
- The pharmacological activity is associated with free-form curcumin.
- Recently, we found that intravenous injection of CMG elevated the concentration of free-form curcumin in the blood in rats.



Multiple sclerosis (MS) and its autoimmune model, experimental autoimmune encephalomyelitis (EAE)

- MS is a chronic immune-mediated disease in the central nervous system (CNS), characterized by inflammation and demyelination (loss of myelin).
- Sensitization of mice with myelin component, such as myelin oligodendrocyte glycoprotein (MOG), induces an MS-like CNS disease; experimental autoimmune encephalomyelitis (EAE).

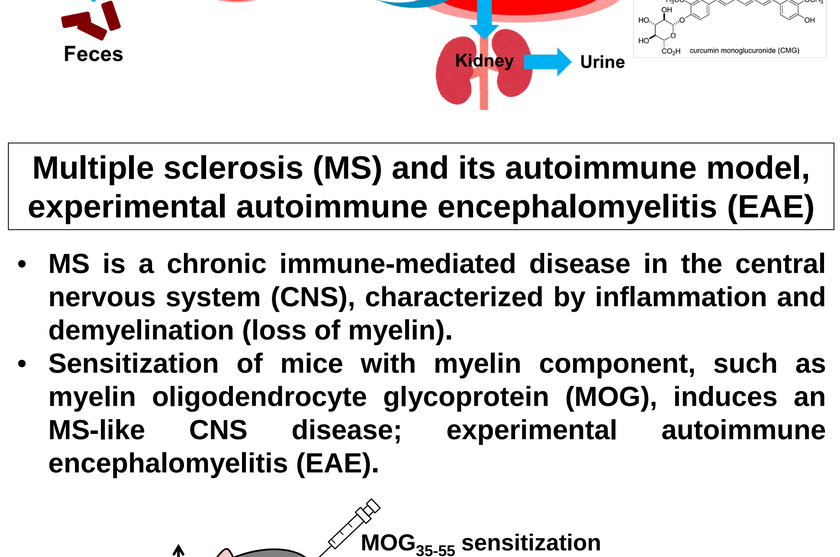


Objective

To determine if CMG treatment affect EAE and microbiota

Results

CMG treatment suppresses EAE



EAE score:
0, no sign
1, tail paralysis
2, hindlimb paresis
3, hindlimb paralysis
4, paraplegia
5, moribund or death

C57BL/6 mice were sensitized twice (days 0 and 19) with myelin oligodendrocyte glycoprotein $(MOG)_{35-55}$ peptide subcutaneously. Moreover, mice were injected with CMG intraperitoneally throughout the whole course or during the induction (days 0-4) or latent (days 11-15) phase. The control mice were injected with PBS. All CMG-treated groups developed less severe EAE compared with the control group.

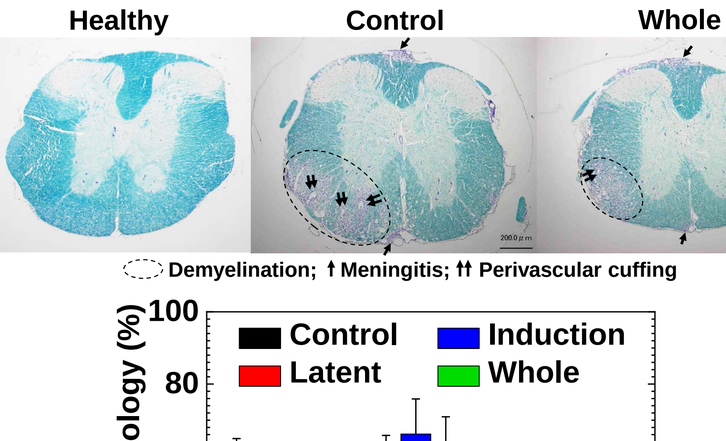
Histological and immunological analyses

CMG treatment suppresses inflammatory demyelination in the spinal cord

Demyelination; † Meningitis; †† Perivascular cuffing

The mouse spinal cord sections were stained with Luxol fast blue. Mice treated with CMG during the latent period or the whole course developed less severe demyelination and inflammation in the meninges (meningitis) and perivascular space (perivascular cuffing) in the spinal cord.

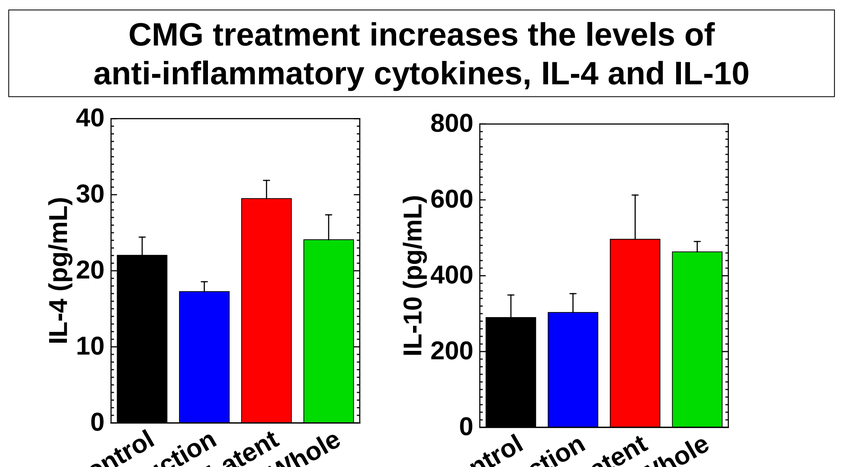
CMG treatment increases the levels of anti-inflammatory cytokines, IL-4 and IL-10



Spleen mononuclear cells were cultured with a mitogen. Cytokine concentration in the culture supernatant was quantified using ELISA kits. CMG treatment enhanced anti-inflammatory IL-4 and IL-10 productions, but not pro-inflammatory IFN-γ or IL-17 production (not shown).

Microbiome analyses

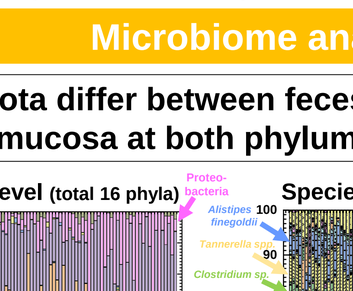
Microbiota differ between feces, ileum content, and ileum mucosa at both phylum and species levels



In both phylum and species levels, the relative abundance of bacteria present in the samples differed between feces, ileum content, and ileum mucosa, regardless of CMG treatment. The microbiota differences dependent on the harvesting sites (feces, ileum content, and mucosa) were larger than the microbiota differences seen among different CMG-treated groups (Control, Induction, Latent, and Whole).

CMG treatment alters the abundance of distinct bacterial species which correlate with EAE scores

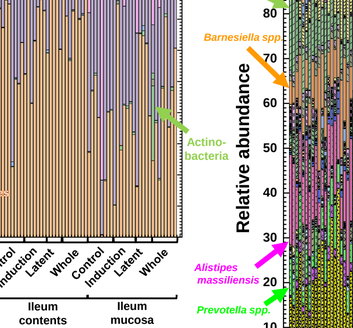
Feces



Species number		
Induction	12	(5↑, 7↓)
Latent	7	(2↑, 5↓)
Whole	20	(4↑, 16↓)

Induction↑ & Latent↑
Enterorhabdus mucosicola
Pseudobutyrivibrio spp.
Induction↓ & Whole↓
Marvinbryantia formatexigens
Syntrophococcus sp.
Latent↓ & Whole↓
Roseburia faecis
Induction↑ & Latent↑ & Whole↑
Blautia Ruminococcus gnavus

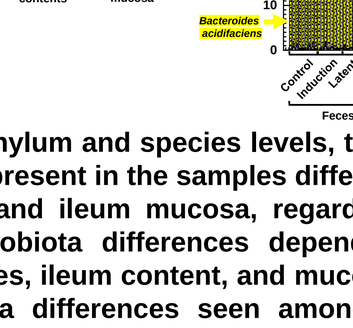
Ileum content



Species number		
Induction	8	(2↑, 6↓)
Latent	3	(1↑, 2↓)
Whole*	14	(6↑, 8↓)

Induction↑ & Whole↑
Parasutterella excrementihominis
Latent↓ & Whole↓
Parabacteroides distasonis
Alistipes putredinis
**Alistipes finegoldii* was decreased in the whole group.

Ileum mucosa



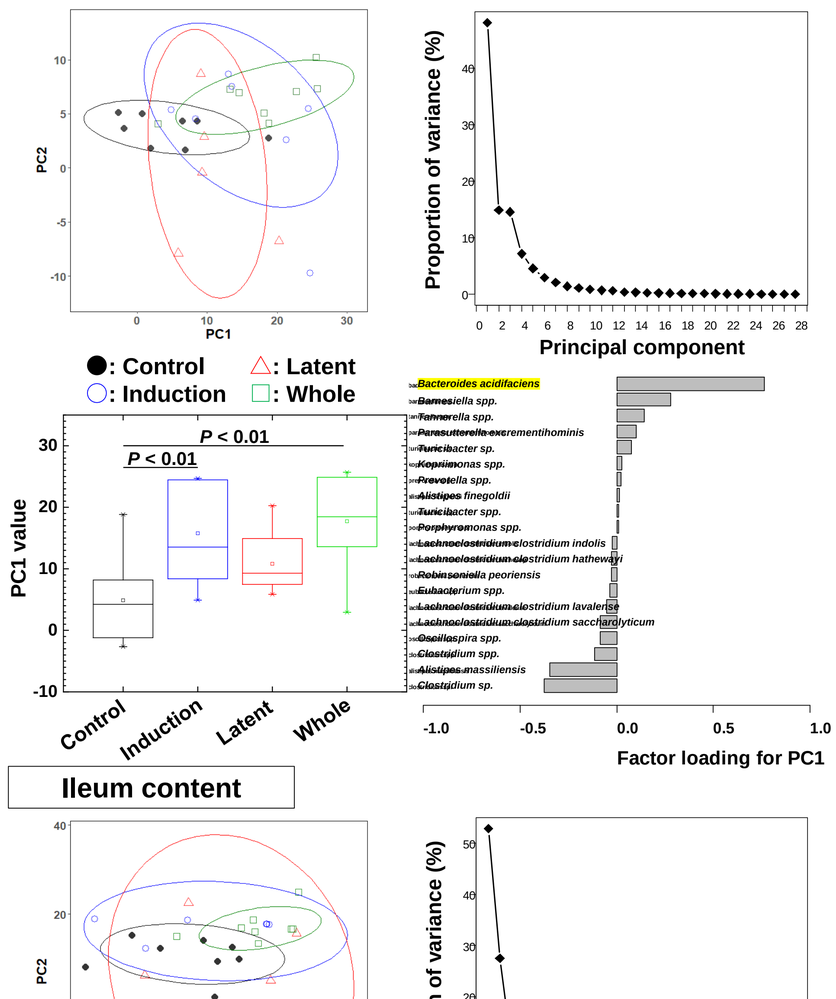
Species number		
Induction	4	(1↑, 3↓)
Latent	1	(1↑)
Whole*	9	(1↑, 8↓)

Latent↓ & Whole↓
Clostridium saccharolyticum
Clostridium perfringens
**Burkholderia spp.* was decreased in the whole group.

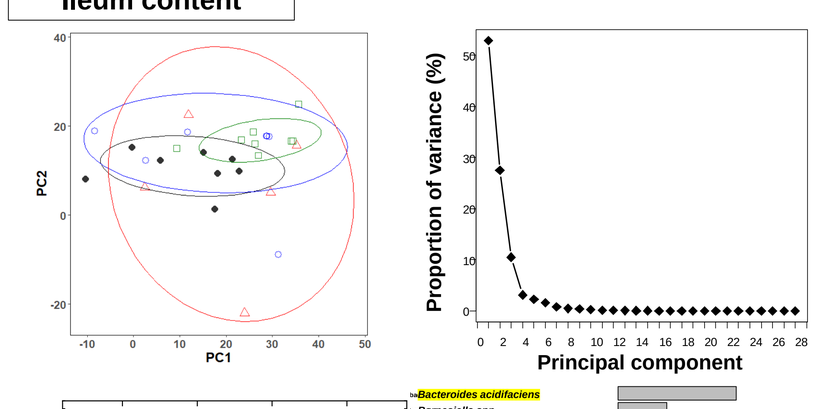
Pattern matching between EAE score and microbiome data was conducted using R. *Blautia Ruminococcus gnavus* in feces, *Alistipes finegoldii* in ileum content, and *Burkholderia spp.* in ileum mucosa most strongly correlated with the EAE scores.

CMG treatment alters microbiota in feces and ileum content but not in ileum mucosa

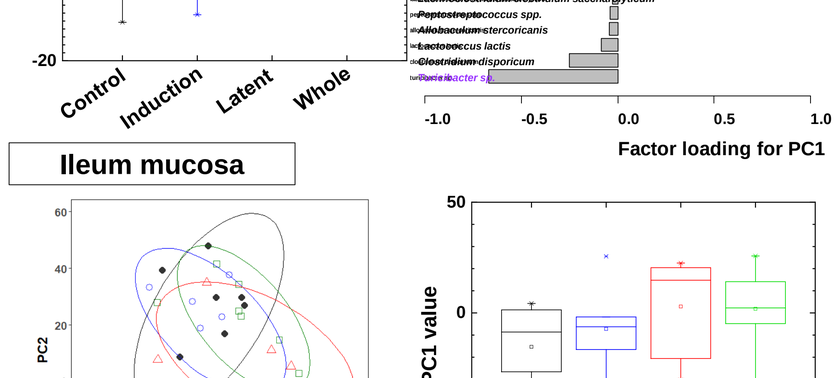
Feces



Ileum content



Ileum mucosa



We conducted principal component analysis (PCA) of the relative abundance of microbiome species. Principal component (PC) 1 values between the control and CMG-treated groups were significantly higher in samples from feces and ileum content, but not ileum mucosa. ***Bacteroides acidifaciens*** correlated PC1 values in feces and ileum content positively; ***Turicibacter sp.*** correlated PC1 values negatively in the ileum content.

PC1 values of the ileum content, but not feces, correlate the clinical and pathology scores in EAE

PC1 values of the ileum content, but not feces, showed a significant correlation with the clinical, demyelination, and inflammation (not shown) scores.

Conclusions

- CMG suppresses EAE, an autoimmune model for MS, clinically and histologically with increased anti-inflammatory cytokines, IL-10 and IL-4.
- CMG treatment alters the abundance of distinct bacterial species in the feces and ileum.
- CMG treatment alters the microbiome in the feces and ileum content, but not in the ileum mucosa.
- The microbiome of the ileum content correlates the clinical and pathological scores of EAE, suggesting that CMG might suppress EAE through alteration of gut microbiota.

References

1) Ozawa, H. et al. (2017). Curcumin β-D-glucuronide plays an important role to keep high levels of free-form curcumin in the blood. *Biol. Pharm. Bull*. 40:1515-1524.
2) Omura, S., Tsunoda, I. et al. (2019). Bioinformatics analyses determined the distinct CNS and peripheral surrogate biomarker candidates between two mouse models for progressive multiple sclerosis. *Front Immunol*. 10:516.

Funding

- Grant-in-Aid for Scientific Research on Innovative Areas “Frontier Research on Chemical Communications” (No 17H06400 and 17H06404, K. Nishio, H. Kakeya, and I. Tsunoda)