

Effect of Administration of Extract from *Coprinus Comatus* on Skin Inflammation induced by UV-B Irradiation

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Abstract

Coprinus comatus is an edible mushroom and contains high amount of ergothioneine, which is a natural amino acid with strong antioxidant activity, anti-photoaging and anti-inflammatory effect. In this study, we examined the inhibitory effects of the extract of *coprinus comatus* on inflammatory responses in mice skin exposed to UV-B.



Coprinus comatus
(*Copri*no Extract- Iwade-51 Strain)

Introduction

Scientific Classification of *Coprinus Comatus*



Coprinus Comatus

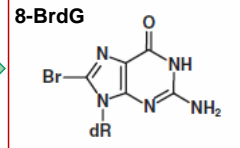
- ◆ contain high amount of **Ergothioneine**
- ◆ have scavenging ability on DPPH radicals, hydroxyl radicals, superoxide radicals

Ergothioneine

- ◆ a naturally occurring amino acid
- ◆ can not be synthesized in the human cells but can be acquired from the diet
- ◆ upon absorption, it distributes in the erythrocytes, lens of eyes and skin
- ◆ antioxidant activity is stronger than ascorbic acid

Inflammatory Biomarker 8-BrdG(8-Bromo-2'-deoxyguanosine)

MPO/H₂O₂/Br⁻
(Myeloperoxidase, MPO)
EPO/H₂O₂/Br⁻
(Eosinophil peroxidase, EPO)



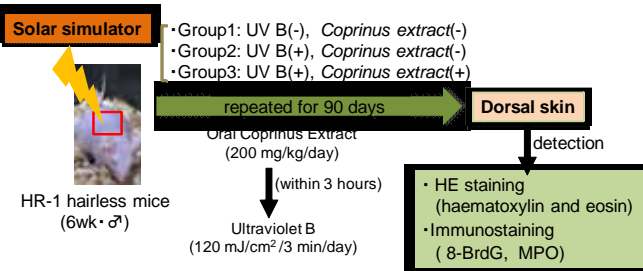
The detection of 8-BrdG Method

Sample	Method
Genomic DNA of rat liver (lipopolysaccharide(LPS)-treated)	Immunohistochemistry LC/MS/MS
Hepatocellular carcinoma (human)	Immunohistochemistry
Urine of human	LC/MS/MS
LPS-treated rat urine	LC/MS/MS

Asahi, T. et al., *J. Bio. Chem.* 285(12), 9282-9291 (2010)

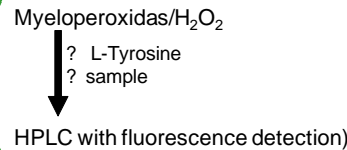
Method-1 (in vivo)

Design of Animal Experiment



Method-2 (in vitro)

The Measurement of MPO Activity



Yoji Kato et al., *Biosci. Biotechnol. Biochem.* 67(5), 1136-1139(2003).

The 8-BrdG Formation in dG/NaOBr Mixture

- 50 mM phosphate buffer(PB)
- 10 mM 2'-Deoxyguanosine(dG)
- Sample or PB(control)
- 1 mM NaOBr
- 37 °C, 1 h
- 10 mM Methionine
- RT, 15 min
- competitive ELISA (with anti-8-BrdG antibody)

Result-1 (in vivo)

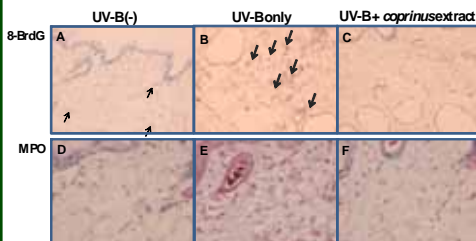
Haematoxylin and Eosin Staining (HE)



Histopathologic changes

- ◆ The thickness of epidermis was increased in UV irradiated mice and was reduced by oral administration of *coprinus* extract (see ↑)
- ◆ Neutrophils were accumulated in the dermis in UV irradiated mice (see ↘)

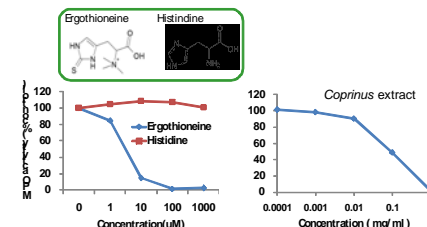
Immunohistochemical Detection



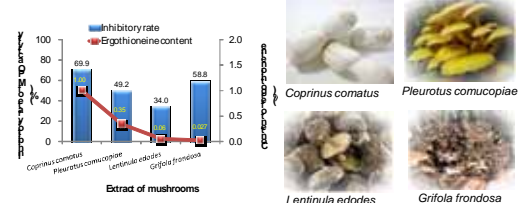
- ◆ Positive immunostainings for MPO and 8-BrdG were observed obviously in dermis compared to non-irradiation control (Fig. B, E).
- ◆ Administration of *coprinus* extract resulted in the reduction of the staining in dermis(Fig.C,F).

Result-2 (in vitro)

Anti-MPO Activity Evaluated by Method-2

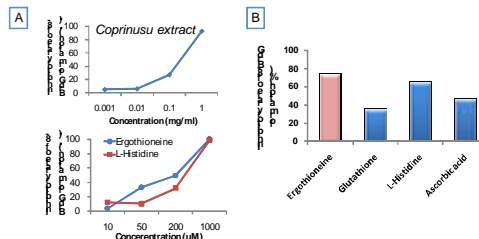


The extract of *coprinus comatus* as well as ergothioneine inhibited MPO activity in a dose-dependent manner. Although the structure of histidine is similar to ergothioneine, it is shown that histidine has no inhibitory effect on MPO activity.



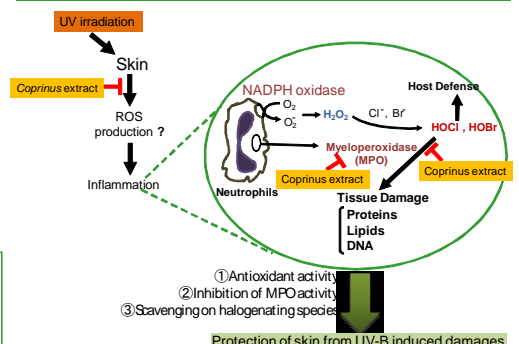
The inhibitory rate of MPO activity of *coprinus* extract is the highest among the four mushrooms examined. It is shown that the inhibitory rate of MPO activity is correlated with the content of ergothioneine.

Inhibitory Effect of *Coprinus* Extract in 8-BrdG Formation Evaluated by Method-2



A: It is shown that *coprinus* extract as well as ergothioneine inhibited halogenation of dG in a dose-dependent manner.
B: Under the same concentration, the inhibitory rate of 8-BrdG formation of ergothioneine was 2.1-fold higher than that of glutathione, and 1.6-fold higher than that of ascorbic acid.

Proposed Mechanism for Protection of *Coprinus* Extract on Skin Inflammation



Conclusions

Although the study regarding metabolism of *coprinus* extract *in vivo* still remains to be examined, these results suggest that the extract of *coprinus comatus* may protect the skin from UV-B induced inflammation, which causes DNA halogenation, through the inhibitions of MPO activity directly and scavenging on halogenous species.