Cationic Dendrimer as a Novel Melanogenesis Inhibitor

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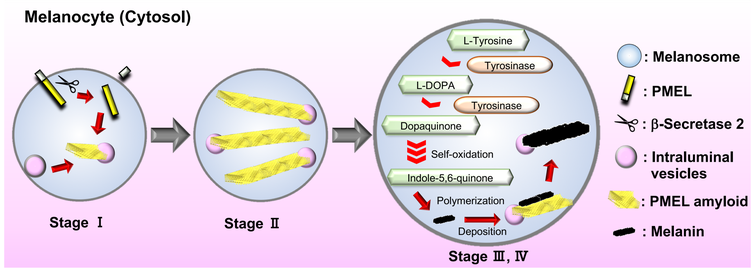
Melanin deposition and whitening agents 1

- **Melanin**, a dark pigment, protects the skin from ultraviolet rays.
- **Amyloidized premelanosome protein (PMEL)** is a scaffold for melanin synthesis.

B. Watt *et al.*, ***Pigment Cell Melanoma Res.,*** 26, 300-315 (2013).

〈 Schematic illustration of intracellular melanin synthesis 〉



B. Watt *et al.*, ***Pigment Cell Melanoma Res.,*** 26, 300-315 (2013).
E. Chuang *et al.*, ***J. Cell Sci.***, 131, 189928 (2018).

Problems of whitening agents

Hydroquinone — Several side effects, Oxidative degradation

Kojic acid — Low whitening effect

A new whitening agent is desired !

Inhibitory effect of a cationic polymer on amyloid formation 2

≪Our previous report≫

1) Starbust® polyamidoamine dendrimer (Generation 2; G2)
2) One of the amyloidgenic protein

Dendrimer (G2) [1)] — **Electrostatic interaction** — **Transthyretin [2)]** → **Suppression of amyloid formation !**

~~~ = $-C_2H_4CONHC_2H_4-$

M. Inoue *et al.*, ***ACS Chem. Neurosci.***, 10, 2584−2590 (2019).

Dendrimer (G2) is expected as a melanin deposition inhibitor through the suppression of PMEL amyloid !

Purpose 3

Evaluation of Dendrimer (G2) as a melanin deposition inhibitor

Outline

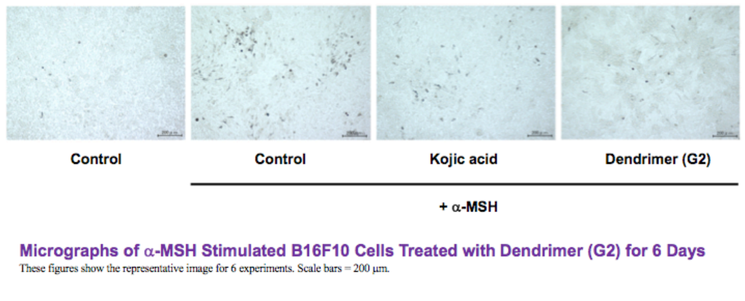
- Inhibitory effect of Dendrimer (G2) on melanin deposition
- Inhibitory effect of Dendrimer (G2) on tyrosinase activity
- Inhibitory effect of Dendrimer (G2) on PMEL amyloid formation
- Cytotoxicity of Dendrimer (G2)

Inhibitory effect of Dendrimer (G2) on melanin deposition 4

1) α-melanocyte stimulating hormone : One of the melanogenic hormones

Kojic acid : 100 μM
Dendrimer (G2) : 50 μM
+ α-MSH [1)]
B16F10 [2)] cells (1.0 × 10^5 cells/well)
6 days, 37°C
Microscopy

2) Mouse melanoma cells

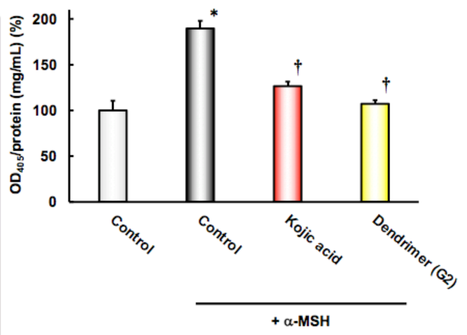


Micrographs of α-MSH Stimulated B16F10 Cells Treated with Dendrimer (G2) for 6 Days

These figures show the representative image for 6 experiments. Scale bars = 200 μm.

Inhibitory effect of Dendrimer (G2) on melanin deposition 5

Kojic acid : 100 μM
Dendrimer (G2) : 50 μM
+ α-MSH
B16F10 cells (1.0 × 10^5 cells/well)
6 days, 37°C
Measurement of protein concentration (BCA assay)
2 h, 65°C
Measurement of melanin absorbance (OD = 405 nm)



Inhibitory Effect of Dendrimer (G2) on Melanin Deposition in α-MSH Stimulated B16F10 Cells

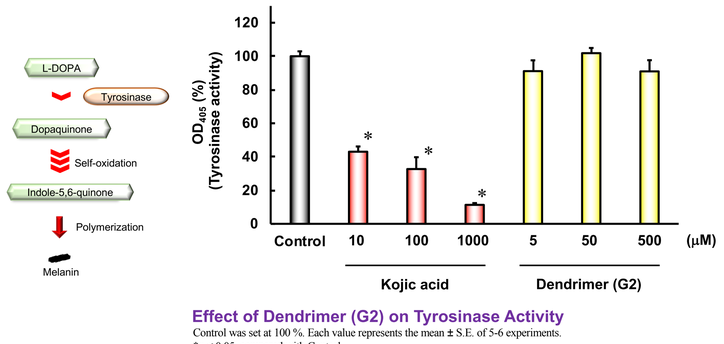
Control was set at 100 %. Each value represents the mean ± S.E. of 5-7 experiments. *$p < 0.05$, compared with Control, †$p < 0.05$, compared with Control + α-MSH.

Dendrimer (G2) inhibited the melanin deposition in B16F10 cells, almost comparable to kojic acid.

Inhibitory effect of Dendrimer (G2) on tyrosinase activity 6

1) The rate-determining enzyme for melanin production

Tyrosinase [1)] (10 units/well)
Kojic acid : 100 μM
Dendrimer (G2) : 50 μM
L-DOPA (0.05 w/v%)
10 min, 37°C
Measurement of melanin absorbance (OD = 405 nm)

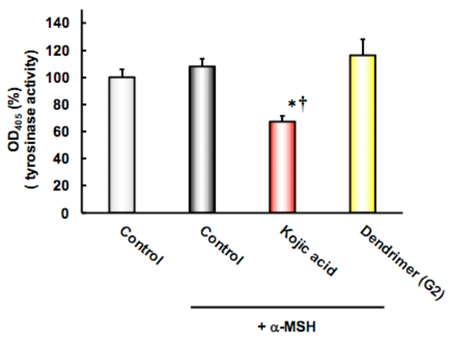


Effect of Dendrimer (G2) on Tyrosinase Activity

Control was set at 100 %. Each value represents the mean ± S.E. of 5-6 experiments. *$p < 0.05$, compared with Control.

Inhibitory effect of Dendrimer (G2) on intracellular tyrosinase activity 7

Kojic acid : 100 μM
Dendrimer (G2) : 50 μM
+ α-MSH
B16F10 cells (1.0 × 10^5 cells/well)
6 days, 37°C
Solubilization
B16F10 cells lysate
L-DOPA (15 mM)
1 h, 37°C
Measurement of melanin absorbance (OD = 405 nm)



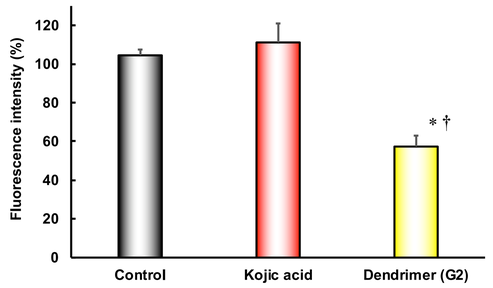
Effect of Dendrimer (G2) on Intracellular Tyrosinase Activity in B16F10 Cells

Control was set at 100 %. Each value represents the mean ± S.E. of 5-6 experiments. *$p < 0.05$, compared with Control + α-MSH, †$p < 0.05$, compared with Dendrimer (G2).

Dendrimer (G2) did not suppress the tyrosinase activity.

Inhibitory effect of Dendrimer (G2) on PMEL amyloid formation 8

PMEL (10 μM)
Kojic acid : 100 μM
Dendrimer (G2) : 50 μM
Citric acid buffer (pH 5.5)
30 min, 37°C
Thioflavin-T assay

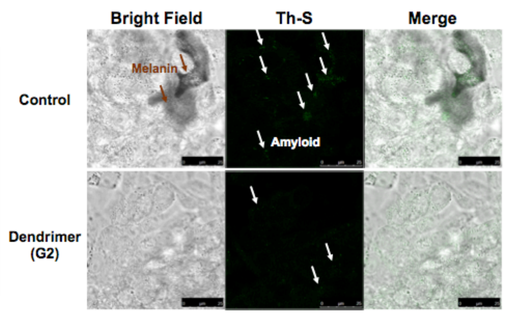


Inhibitory Effect of Dendrimer (G2) on PMEL Amyloid Formation

Each value represents the mean ±S.E. of 5-6 experiments. *$p < 0.05$, compared with Control, †$p < 0.05$, compared with kojic acid.

Inhibitory effect of Dendrimer (G2) on intracellular PMEL amyloid formation 9

Dendrimer (G2) : 50 μM
B16F10 cells (1.0 × 10^5 cells/well)
6 days, 37°C
Thioflavin-S staining (Fluorescence microscopy)



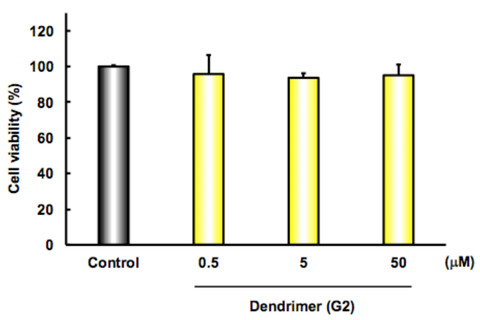
Inhibitory Effect of Dendrimer (G2) on Intracellular Amyloids in B16F10 Cells

Brown arrows indicate the melanin. White arrows indicate intracellular amyloids. These figures show the representative image for 5-7 experiments. Scale bars = 25 μm.

Dendrimer (G2) inhibited PMEL amyloid formation.

Cytotoxicity of Dendrimer (G2) in B16F10 cells 10

Dendrimer (G2) : 50 μM
B16F10 cells (1.0 × 10^5 cells/well)
6 days, 37°C
Cytotoxicity assay (WST-8)

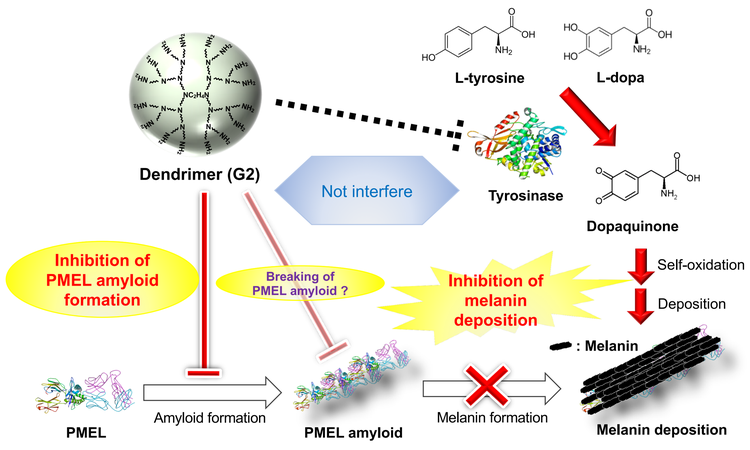


Cytotoxicity of Dendrimer (G2) in B16F10 Cells

Control was set at 100 %. Each value represents the mean ± S.E. of 3 experiments.

Dendrimer (G2) did not induce cytotoxicity in B16F10 cells up to 50 μM.

Proposed mechanism of the whitening effect by Cationic Dendrimer (G2) 11



Dendrimer (G2) may inhibit intracellular melanin deposition through the inhibition of PMEL amyloid formation !

Conclusion 12

- Dendrimer (G2) inhibited the melanin deposition in B16F10, almost comparable to kojic acid.
- Dendrimer (G2) did not suppress the tyrosinase activity.
- Dendrimer (G2) inhibited PMEL amyloid formation.
- Dendrimer (G2) did not induce cytotoxicity in B16F10 cells up to 50 μM.

Dendrimer (G2) may have a potential as a melanogenesis inhibitor through the inhibition of PMEL amyloid formation.

Acknowledgements

Department of Analytical and Biophysical Chemistry, Kumamoto University
Dr. Yoshihiro Kobashigawa
Dr. Hiroshi Morioka
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