靈芝子實體細胞壁組成 SACCHACHITIN 對 B16 細胞黑色素形成抑制之探討

Inhibition of Melanogenesis in B16 cells by SACCHACHITIN, a Cell Wall Component from Ganoderma tsugae

中文摘要

本研究以 SACCHACHITIN（靈芝多醣幾丁質）進行研究，探討其對酪胺酸?活性的影響。由於之前已證實此材料能有效抑制蛋白質水解酵素（如 MMP-1，MMP-8，MMP-9）以及脂肪?（Lipase），所以本實驗將進一步探討其抑制酪胺酸?活性之成效。本研究首先探討對抑制蕈酪胺酸? （Mushroom tyrosinase）活性的影響性，結果顯示：2.5 mg/ml 的抗壞血酸顯示出非常顯著的抑制作用，在不同來源的幾丁聚醣中，只有 SACCHACHITIN 呈現劑量依存反應（dose-dependent reaction）其 IC50 約爲 17.2 mg/ml。接著在細胞模式下，SACCHACHITIN 對 B16 細胞型態和增生沒有不良影響，不具有細胞毒性。再進行 B16 細胞黑色素含量的分析，加入誘導劑 α -MSH 濃度爲 25 nM 所增加的百分比率最高，可以提高 3.37 倍的黑色素含量。不論在無或有誘導劑之下，SACCHACHITIN 均可以減少 B16 細胞產生黑色素，和降低 B16 細胞之酪胺酸?活性，呈現劑量依存反應（dose-dependent reaction）。西方點墨法再次確認 SACCHACHITIN 確實能夠抑制 B16 細胞中酪胺酸?蛋白質的表現，尤其是當 SACCHACHITIN 濃度爲 2 mg/ml 時，效果更加顯著，同時且隨著 SACCHACHITIN 的微粒化，有增進抑制 B16 細胞中酪胺酸?蛋白質表現的結果。

英文摘要

SACCHACHITIN was prepared from the waste residue of the fruiting body of Ganoderma taugae, was used in our previous study to inhibit matrix metalloproteinases and lipase. In the present study, SACCHACHITIN was estimated for its effects on the tyrosinase activity and expression. In the mushroom tyrosinase inhibition assay, the results indicated that 2.5 mg/ml of ascorbic acid had strong inhibition of mushroom tyrosinase. In different source of chitin, only SACCHACHITIN showed a potent tyrosinase activity inhibition, IC50 value was 17.2 mg/ml. In the cell culture assay, SACCHACHITIN showed neither morphological alteration nor disorder in proliferation. These evidences elucidated that SACCHACHITIN was not cytotoxic and safe toward the growth of B16 mouse melanoma cells. Use 25nM -MSH induce melanogenesis in B-16 cells could elevate 3.37 times of melanin content. The results of inhibitory effect of

SACCHACHITIN with no inducer and with inducer -MSH on melanin biosynthesis and tyrosinase activity were observed in B-16 mouse melanoma cells all in a dose-dependent response reaction.

Western blot was employed to ensure SACCHACHITIN inhibited B16 cells tyrosinase protein expression, and the results indicated that 2.0 mg/ml SACCHACHITIN had obviously inhibitary effect. In the same experiment, SACCHACHITIN in small particle (average size: 10 μm) seemed in clined to enhance the inhibition of tyrosinase protein expression.