蜂王漿之生物活性及其相關研究

Studies on the biolgical activity in royal jelly

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[摘要]

蜜蜂因食用蜂王漿而變為蜂后，蜂王漿也被認為具有抗老化，使人恢復活力，返老還童之功效。在民間食物療法中，蜂王漿具有免疫調節、免疫刺激作用，可以增加對疾病的抵抗力，並可調節中樞神經系統系統，而具有良好的抗老化作用。從這些未經證實的民間體驗中，可以初步的推論蜂王漿內可能含有對中樞神經系統及免疫系統有作用生物活性物質。 本研究採用了四種生物篩選模式來研究蜂王漿是否具有生物活性物質。 在免疫系統方面，首先採用老鼠顆粒球/單核球幹細胞系統在低濃度 L929 條件培養液下進行 初步篩選，發現粗萃取的蜂王漿對 DNA 約合成約有 1.4 倍的促進作用。為使此系統更加單純化，進一步使用重組技術，於大腸桿菌內大量表現 mGM-CSF，使用陰離子交換樹脂層析法及 PrepCell 純化得到分子量為 14.lkTa 的蛋白質去免疫兔子得到多株抗體，利用純化具有活性 之 2ne/ml mGM-CSF 來取代 L929 條件培養液。在此新系統下，發現大部分經由 HW55F 管柱 分出的區分多具有從進其 DNA 的合成的作用，再進一步經由 LH-20，silica 管柱再分離有效 成分，最後分離出純化之 F5L1ISI 在 160ne／mll 濃度下對骨髓細胞 DNA 有 1.35 倍的促進作用 。經由 1'H-NMR 及 3C-NMR 結構分析，F5LIS1 可能為一寡醣物質。免疫系統方面的另一篩選系 統以 Concannavalmn A 活化之老鼠脾臟免疫細胞，結果顯示大部分經由 HW55F 分出的部份多 具有抑制其 DNA 的合成的作用，但再經由 LH-20 分離後的成分 F6L2, F6L3, F6L4, F6L5 則具 有 DNA 合成促進作用的 1.2 倍。 在中樞神經系統方面，首先由大白鼠十七天至十八天的胚胎取大腦皮質培養腦皮質細胞， 在不完全的 N2 培養液加入 1%的馬血清下，先進行篩選。另一方面則繼續培養液的調整以建 立無血清(serum free)的培養模式。在不完全的 N2 培養液模式下，一些經由 HWV55F 管柱分 出區分對腦皮質細胞具有 DNA 合成促進作用。而在不具血清的篩選模式下，篩選出 F5，F6 具 有增加腦神經幹細胞 DNA 合成 1.4 及 1.2 倍的作用，F7 則具有抑制的效果。經由進一步使用 LH-20，silica 管柱繼續分離。最後得到 F5LIS1 及 F5LIS2 純化物質具有 DNA 合成促進作用。 經由 1'H－NMR 及 13C－NMR 的結構分析，F5LIS1 可能為一寡醣物質，F5LIS2 可能為一醣胜汰 。而在星狀細胞方面，初步觀察之結果顯示蜂王漿之成分多為抑制其 DNA 合成。 本研究的結果顯示蜂王漿內含有對免疫及神經系統具生物活性的物質。純化之 F5L1S1 及 F5L1S2 分別為寡醣及醣胜汰且其結構和作用機轉非常值得進一步研究。

關鍵字: 生物科技 BIOTECHNOLOGY、藥學 PHARMACOLOGY
蜂王漿、生物活性

Royal jelly is the food on which bees are fed to develop into queen bees. It is claimed that royal jelly has anti-aging and rejuvanting virtues forhuman beings. As a folk food therapy, royal jelly has profound effect onanti-aging through modulating CNS function, increasing resistance to disease and promoting body immunity. According to these primitive observations, it's predicted that royal jelly exist bioactive compounds acting on CNS andimmune systems of human. This report describes the screening of the activecompounds in royal jelly in four bioassay systems. In immune system, using mouse GM (Granulocyte Monocyte)-stem cell modelsystem with low L929 conditioned medium, crude extracts of royal jelly increasedDNA synthesis for about 1.4 folds. For simplifying the bioassay, the L929 conditioned medium was replaced by mGM-CSF in Escherichia coli. The overexpressed mGM- CSF was purified through anion-exchange chromatographyand Prep Cell. A 14.1 kDa protein was obtained and the polyclonal antibodywas immunized in rabbit. Using purified mGM-CSF at a concentration of 2 ng/mlto replace L929 conditioned medium, it was found that some HW55F fractions stimulated DNA synthesis. Further, after LH- 20 and silica column to subfractionate the active fractions, a pure compound, F5L1S1, wasobtained. It stimulated DNA synthesis for about 1.35 folds at a concentration of 160ng/ml. Its structure was characterized by 1H-NMR and 13C-NMR. Structure analysis indicated this compound might be a oligosaccharide derivative. InConcanavalin A activated splenocytes screening system, F6L2, F6L3, F6L4 and F6L5fractions also stimulated DNA sysnthesis for about 1.2 folds. In central nervous system, by using cultured cortex cells from E17-18 rat embryoes with 1% horse serum, some HW55F fractions from royal jelly wasfound to increase DNA synthesis of these cells. When serum free cortex cellsculture system was used, the subfractions F5 and F6 increased its DNA synthesisabout 1.4 and 1.2 folds. After further purification by LH-20 and silica gel chromatography, F5L1S1 and F5L1S2 were obtained as pure compound with much higher activity. These compounds were analysed by 1H-NMR and 13C-NMR.The structure analysis suggested that F5L1S1 might be a oligosaccharide andF5L1S2 might be a glycopeptide. In astrocyte bioassay, most HW55F fractionsshowed inhibitory effect on DNA synthesis of these cells. The results indicated that royal jelly contained bioactive substanceswhich effect on immune and nerve system. F5L1S1 and F5L1S2 were purified and identified as oligosaccharide and glycopeptide. Furteher structureanalysis and mechanism of these compounds are worth of further studies.