Luteolin 在活體及離體抗氣喘的作用機轉

Mechanisms of anti-asthmatic action of Luteolin in vivo and in vitro

中文摘要

第一部分:

Flavonoids 是一種天然的 polyphenolic compounds，廣泛的存於植物中，有抗發炎和免疫調控的功能。Cyclic nucleotides 在發炎細胞扮演十分重要的角色，而氣道平滑肌的舒張也與細胞內 cyclic nucleotides 濃度有關。我們有興趣研究 flavonoids 對於來自天竺鼠心臟及肺臟之 PDE 各種亞型(isozyme)的抑制效果，和其作用¾結構的關係。

天竺鼠的肺臟及心臟經研磨及離心，使上清液通過 Q-Sepharose 陽離子交換樹脂，藉著改變 NaCl 的濃度，便可依序由肺臟分離得到 PDE1、PDE5、PDE2 及 PDE4，而由心臟得到 PDE3。

根據 Thompson 及 Appleman 的方法，利用 cAMP 與 [3H]-cAMP 或 cGMP 與 [3H]-cGMP 作為 PDE 的受質，測定 PDE 活性。結果顯示 luteolin 對 PDE1~5 均具有效的抑制作用，若在其 C-4'' OH 基以 OCH3 基取代成 diosmetin，則會增加對 PDE2、但喪失對 PDE3 抑制作用，若去掉 C-3'' 之 OH 基而成 apigenin，則喪失對 PDE4, 5 的抑制作用，若去掉 C-3''、4'' 之 OH 基而成 chrysin，則喪失對 PDE1~5 的抑制作用，若 luteolin 醣化成 luteolin -7-glucoside，則降低對 PDE2、4，甚至於喪失對 PDE1、3、5 的抑制作用，若 luteolin 在 C-3 加入 OH 基成 quercetin，則增加對 PDE4，但喪失對 PDE5 的抑制作用，若在 C-3、C-5''各加入 OH 基成 myricetin，則減少對 PDE4，但喪失對 PDE5 的抑制作用，若 luteolin 在 C-2, 3 間的雙鍵變單鍵成 eriodictyol，則減少對 PDE3，但喪失對其他 PDE 的抑制作用，若 diosmetin 在 C-2, 3 間的雙鍵變單鍵成 hesperetin，則減少對 PDE4，但喪失對其他 PDE 的抑制作用。

第二部分:

Luteolin 能有效地抑制 PDE1~ 5，但不具選擇性，是否有抗氣喘的作用，是本篇實驗的目的。在氣道過度反應的活體實驗中，我們利用整體體積描述器來分析氣管收縮的情形，結果顯示 luteolin 能抑制卵蛋白(OVA)引起的氣道過度反應(AHR)，luteolin (3~30 mmol/kg, i.p.)亦能劑量依存性地減少 MCh (25~50 mg/ml)引起的 Penh 值增加，luteolin 30 mmol/kg 甚至於有意義地抑制 MCh (12.5 mg/ml)的 Penh 值，投與 luteolin (3-30 mmol/kg)的小白鼠與敏感化未暴露於 OVA 氣化噴霧之小白鼠(non-treatment)無異。觀察肺泡灌流液(BALF)的細胞沉澱物，發現 luteolin (3-30mmol/kg) 也有意義地抑制總發炎細胞數、嗜中性血球及嗜伊紅白血球，但非淋巴球，劑量達 30 mmol/kg 更能有意義地減少巨噬細胞。Luteolin (3-30 mmol/kg)有意義地降低在 BALF 中 IL-2， IL-4， IFN-g，和 TNF-a 的釋放，劑

量達 30 mmol/kg 更有意義地抑制 IL-5 的釋放。Luteolin (3~30 mM)能有意義地降低 OVA (10 mg/ml)引起之離體天竺鼠氣管的收縮。由 Lineweaver-Burk 分析發現 luteolin (3~30 mM)對各種 PDE isozymes 呈競爭性的抑制。結論，luteolin 能非選擇性但競爭性地抑制 PDE1~5，低劑量(3-30 mmol/kg, i.p.)具抗發炎及支氣管擴張作用，可能有治療氣喘的潛力。

英文摘要

Part I:

Flavonoids are polyphenolic compounds occurring in nature, commonly presents in plants. They have anti-inflammatory and immuno-regulatory effects. Cyclic nucleotides play an important role in inflammatory cells. Their concentration is related to the relaxation of airway smooth muscle. Therefore we are interested in investigating the relationships between structure and inhibitory effect of flavonoids on various PDE isozymes separated from guinea-pig lungs and hearts.

Isolated guinea-pig lungs and hearts were separately homogenized and centrifuged. The supernatant was chromatographed over a column of Q-sepharose, and eluted with various concentrations of NaCl. In the following order, PDE subtype 1, 5, 2, 4 from lungs, and 3 from hearts were separated.

Acccording to the method described by Thompson and Appleman, the activities of PDE isozymes were determined in the presence of cAMP and [3H]-cAMP or cGMP and [3H]-cGMP as substrate. The results revealed that luteolin had effectively inhibitory actions on PDE1~5. Diosmetin, which OCH3 group substitutes the C-4 OH group of luteolin, enhanced inhibition on PDE2, but lost inhibition on PDE3 activity. Apigenin, lacking of C-3'' OH group from luteolin, lost inhibition both on PDE4 and 5. Chrysin lacking of both C-3'' and C-4'' OH groups from luteolin lost all inhibition on PDE1~5. Luteolin-7-glucoside glycosylated from luteolin attenuated inhibition on PDE2 and 4, lost inhibition on PDE1, 3, and 5. Quercetin hydroxylated from luteolin at position of C-3 enhanced inhibition on PDE4, but lost inhibition on PDE5. Myricetin, hydroxylated from luteolin at both position of C-3 and C-5'', enhanced inhibition on PDE4, but lost inhibition on PDE5. Eriodictyol saturated luteolin between C-2 and 3 attenuated inhibition on PDE3, but lost inhibition on other PDE isozymes. Hesperetin saturated from diosmetin between C-2 and 3 attenuated inhibition on PDE4, but lost inhibition on other PDE isozymes.

Part II:

Luteolin effectively but non-selectively inhibited PDE1~5. The aim of this present study is to investigate whether it has anti-asthmatic action. The present results revealed that luteolin inhibited ovalbumin (OVA)-induced airway hyperresponsiveness (AHR). Luteolin (3-30 mmol/kg, i.p.) dose-dependently attenuated the enhanced pause (Penh) value induced by aerosolized methacholine (MCh, 25~50 mg/ml) in sensitized mice after secondary allergen challenge. Luteolin at 30 mmol/kg even significantly inhibited MCh (12.5 mg/ml)-induced increase of Penh value. Mice administered luteolin (3-30 mmol/kg) did not significantly differ from those sensitized but not challenged with aerosolized OVA (non-treatment). Luteolin (3-30 mmol/kg, i.p.) also suppressed total inflammatory cells, neutrophils and eosinophils, but not lymphocytes. Luteolin at a dose of 30 mmol/kg even significantly reduced marcrophages. Luteolin (3-30 mmol/kg) significantly reduced the IL-2, IL-4, IFN-g and TNF-a production in bronchoalveolar lavage fluid (BALF). At a dose of 30 mmol/kg, luteolin even inhibited release of IL-5. Luteolin (3~30 mM) significantly attenuated OVA (10 mg/ml)-induced tracheal contractions in vitro. From Lineweaver-Burk analysis, luteolin (3~30 mM) competitively inhibited various PDE isozymes. In conclusion, luteolin non-selectively but competitively inhibited PDE1~5. At low doses of 3~30 mmol/kg (0.858~8.58 mg/kg), luteolin has both anti-inflammatory and bronchodilatory effects, and has a potential in the treatment of asthma.