Genistein 在活體及離體抗氣喘的作用機轉

Mechansims of anti-asthmatic action of genistein in vivo and in vitro

中文摘要

第一部份

本篇論文嘗試探討 isoflavones 抑制 PDE isozymes 之活性與結構的關係，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 1971 年 Thompson 及 Applemen 的方法，利用 cAMP 與 [3H]-cAMP 或 cGMP 與 [3H]-cGMP 作爲 PDE 的受質，測定 PDE 活性。結果顯示在這些 isoflavones 中，以 genistein 對 PDE2 的作用最強，其次對 PDE1、3、4，但對 PDE5 幾無作用。Daidzein 是構造類似 genistein，但對 protein tyrosine kinase 不具活性的物質，對 PDE3 具選擇性的抑制作用。Biochanin A 對 PDE4 的抑制作用最強，其次是對 PDE1 及 PDE2，但對 PDE3 及 pDE5 無抑制作用。Prunetin 對所有 PDE isozymes 均無作用。由於這些 isoflavones 的構造均極接近，因此我們綜合結論 C-7 的 OH 基對 PDE 1, 2, 3, 4, 5 皆很重要，C-4''的 OH 基對 PDE 3, 5 很重要，其次對 PDE 1, 2 也重要，C-5 的 OH 基對 PDE 1, 2, 4, 5 很重要，其次對 PDE 3 也重要。

第二部份

Genistein 爲一知明廣效的 protein tyrosine kinase (PTK) 抑制劑，包括 genistein 在內的這些 isoflavone 自然存在於黃豆中，已有許多報導指出 isoflavone 有益健康，有保護心臟、抗氧化、抗免疫及抗發炎的作用，因此我們有興趣研究 genistein 在活體及離體抑制卵蛋白(OVA)所引發的氣道過度反應。根據 2001 年 Kanehiro 等人的方法，腹腔內注射 OVA 於雌 BALB/c 鼠，使其主動產生敏感，並以氣化 OVA (1 %)挑戰之，第二次挑戰後，氣化 methacholine (MCh, 6.25-50 mg/ml)會使這些清醒動物之 enhanced pause (Penh)值，濃度依存性地增加， genistein (10~100 mmol/kg, i.p.) 明顯抑制這些敏感化小白鼠因 MCh(50 mg/ml)引起的 Penh 值增加。此外，genistein 有意義地減少肺泡灌流液中的總發炎細胞、巨噬細胞、嗜中性白血球及嗜伊紅白血球的增加,但非淋淋巴球,並且也會明顯降低 cytokines 包括 IL-2, IL-4, IL-5, IFN-g 及 TNF-a 的增加，唯一例外是 genistein 在 30 mmol/kg 無意義地抑制 INF-g 的增加。Genistein (3~30 mM)有意義地抑制 OVA 引起的過敏性天竺鼠離體氣管的收縮。

Genistein 選擇性並競爭性地抑制 PDE2 及 3,其 Ki值低,分別爲 4.32 及 11.47 mM， 其次抑制 PDE1 及 4，但對 PDE5 無作用。綜合以上結果，genistein 抗氣喘之可能途徑是經由抑制 cAMP-PDE，而非抑制 PTK 活性而來，因抑制

PTK 活性須要更高濃度。

英文摘要

PART 1

In this present study, we tried to investigate that the structure-activity relationships between isoflavones and their inhibitory effects on PDE isozymes. 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 partially purified. Acccording to the method described by Thompson and Appleman in 1971, 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 in these isoflavones, genistein most potently inhibited the activity of PDE2, moderately inhibited the activities of PDE1, 3 and 4. However, genistein did not inhibit the activity of PDE5. Daidzein, an inactive analog of genistein, which has been reported to specifically inhibit protein tyrosine kinase, selectively inhibited the activity of PDE3. Biochanin A potently inhibited the activity of PDE4, moderately inhibited the activities of PDE1 and 2, but never inhibited the activities of PDE3 and 5. Prunetin almost did not inhibit these five PDE isozymes. Based on the similarity of structures of these isoflavones, therefore, we conclude that C-7 OH group is very important for PDE1~5, that C-4¢ OH group is very important for PDE3 and 5, subsequently for PDE1 and 2, and that C-5 OH group is very important for PDE1, 2, 4 and 5, subsequently for PDE3.

PART 2

Genistein is a well known broad-spectrum protein tyrosine kinase (PTK) inhibitor. These isoflavones including genistein, are naturally occurring in the legume soybean. There have been numerous reports of isoflavones about their beneficial health effects, such as cardioprotection , antioxidant, anti-immune response, and anti-inflammation. Therefore we are interested in investigating its suppressive effects on ovalbumin (OVA)-induced airway hyperresponsiveness in vivo and in vitro. According to the method described by Kanehiro et al., in 2001, female BALB/c mice were actively sensitized by intraperitoneal injections of OVA and challenged by aerosolized OVA (1 %). After secondary challege, aerosolized methocholine (MCh, 6.25-50 mg/ml) induced increases of enhanced pause (Penh) values in conscious animals in a concentration-dependent manner. Genistein (10~100 mmol/kg, i.p.) markedly inhibited MCh (50 mg/ml)-induced increase of Penh value in the sensitized mice. In addition, genistein significantly reduced increases of total inflammatory cells, macrophages, neutrophils, and eosinophils, but not lymphocytes in bronchoalveolar

lavage fluid, and markedly attenuated the release of cytokines, including IL-2, IL-4, IL-5, IFN-g and TNF-a with an exception that genistein at 30 mmol/kg did not significantly inhibit INF-g. Genistein (3~30 mM) significantly inhibited OVA-induced contrations in sensitized guinea pig isolated trachea.

Genistein selectively and competitively inhibited PDE2 and 3, with a low Ki value of 4.32 and 11.47 mM, respectively. It subsequently inhibited PDE1 and 4, but not 5.

The above results revealed that anti-asthmatic action of genistein is via inhibition of cAMP-PDE but not PTK activity, which has been reported to need higher concentration to inhibit.