以氣相層析質譜儀分析氧化壓力指標-水楊酸鹽產物並評估多酚性化合物清除氫氧自由基之能力

Using Salicyate Adducts as an Oxidative Stress Maker by GC/MS and Evaluate Hydroxyl Radicals Scavenging Effect of Polyphenols

中文摘要

在所有有氧生物體中，經由呼吸爆炸 (respiratory burst)所產生之超氧化陰離子自由基(superoxide anion radical)和過氧化氫(hydrogen peroxide)，在螯合鐵存在下，進行 Fenton’s 反應，產生氫氧自由基(•O H)。它是最具活性的氧自由基，以 107-1010 M-1S-1 的擴散速率(diffusion control rate)與大多數生物分子作用，引起細胞膜脂質過氧化反應(membrane lipid peroxidation)，蛋白質凝集(protein aggregation)和 DNA 羥化反應(hydroxylation)，因而瓦解細胞的功能和完整性。由於氫氧自由基的半衰期短和反應性高，不易直接偵測，目前偵測體內氫氧自由基最有效且具特異性的方法之一是利用芳香環上羥化(aromatic hydroxylation)的方法。以水楊酸鹽(salicylate)與氫氧自由基經加成反應產生之羥化物，被廣泛的應用在體外或體內試驗，以偵測氫氧自由基之生成或評估具氫氧自由基清除作用的效力。

在體外試驗，以 Fenton’s 試劑所產生之氫氧自由基與 salicylate 反應，其生成物(2,3-DHBA 及 2,5-DHBA)以氣相層析質譜分析方法測定，並評估酚性化合物(如類黃鹼素；flavonoids)、維他命 C、E、抗氧化劑(如 probucol)等測試物之清除氫氧自由基的能力，其大小依序爲 quercetin (55.8%)，rutin (54.7%)，trolox (51.1%)，thiourea (50.1%)，myricetin (48.1%)，luteolin (45.7%)，(-)-epigallocatechin (41.9%)，(-)-epicatechin (41.7%)，morin (41.3%)，(+)-catechin (40.6%)， (+)-gallocatechin (38.8%)，kaempferol (38.1%)，wogonin (31.8%)，liquiritin (30.9%)，bacailein (30.2%)，DMSO (30.2%)，capillarisn (27.3%)，genistein (26.9%)，probucol (26.9%)，β-carotene (26.6%)， ascorbic acid (-)，d-α-tocopherol (-)。

在體內試驗中，先以腹腔注射 salicylate，觀測兔子體內受 1,1,2,2-tetrachloroethane 抑制 cytochrome P450 後，視其羥化產物 2,3-DHBA 及 2,5-DHBA 生成量之變化。

英文摘要

All aerobic organisms produced superoxide anion and hydrogen peroxide via respiratory burst. In the presence of chelated iron, these species are converted to hydroxyl radicals through a Fenton reaction . The hydroxyl radical is an extremely reactive oxidizing radical that will react with most biomolecules at diffusion-controlled rates (107-1010 M-1S-1), and cause membrane lipid peroxidation, protein aggregation, and DNA hydroxylation, thereby disrupting cellular functions

and integrity.
Because hydroxyl radical has a very short half-life and high reactivity, its measurement is very difficult. The trapping method with potential for specifically identifying hydroxyl radicals is aromatic hydroxylation. The method of salicylate produced hydroxylation adducts (2,3-DHBA and 2,5-DHBA) with hydroxyl radicals, was large used to assessment of the scavenging activity of hydroxyl radical both in vitro and in vivo.
In vitro study, the polyphenols and others scavenged hydroxyl radicals generated by Fenton reaction. Free hydroxyl radicals were trapped by salicylate and adducts was detected by GC/MS. The scavenging activity of polyphenols decrease in the order: quercetin (55.8%) ， rutin (54.7%)， trolox (51.1%)，
thiourea (50.1%)， myricetin (48.1%)， luteolin (45.7%)，(-)-epigallocatechin (41.9%)，(-)-epicatechin (41.7%)， morin (41.3%)，(+)-catechin (40.6%)，(+)-gallocatechin (38.8%)，kaempferol (38.1%)，wogonin (31.8%)，liquiritin (30.9%)，bacailein (30.2%)，DMSO (30.2%)，capillarisn (27.3%)，genistein (26.9%)，probucol (26.9%)，β-carotene (26.6%)，ascorbic acid (-)，d-α-tocopherol (-)。
In vivo study, the change of the amount of 2,3-DHBA and 2,5-DHBA were observed when the rabbits were treated with IP of salicylate and induced by 1,1,2,2-tetrachloroethane to inhibit cytochrom P450.