銀杏葉萃取物體外與體內實驗對肝癌之影響

The effects of Ginkgo biloba extract on hepatocellular carcinoma in vitro and in vivo

中文摘要

本研究之目的爲探討銀杏葉萃取物標準製劑(EGb761:含 22-27%銀杏黃酮配醣體與 5-7%類苕烯)，對肝癌細胞之影響。體外實驗部份，添加不同劑量(0-1000 mg/mL)之 EGb761 於人類肝癌細胞株(HepG2 與 Hep3B2.1-7)培養液中，培養 24~48 小時後分析 HepG2 與 Hep3B2.1-7 細胞之增殖情形、乳酸脫氫酶外漏比率、HepG2 細胞其 p53 蛋白質與增殖細胞核抗原(proliferating cell nuclear antigen; PCNA)之表現量。體外實驗結果顯示：添加 EGb761 濃度爲 50-1000 mg/mL，皆可明顯抑制 HepG2 與 Hep3B2.1-7 細胞之增殖，並增加乳酸脫氫酶外漏比率。添加 EGb761 (1000 mg/mL)於 HepG2 與 Hep3B2.1-7 細胞之抑制增殖情形相較於控制組(無添加 EGb761)分別爲 45.3%與 38.8% ($p < 0.05$)。乳酸脫氫酶外漏比率於 HepG2 細胞之控制組與添加 EGb761 (1000 mg/mL)組分別爲 6.7%、37.7%，於 Hep3B2.1-7 細胞則分別爲 7.2%、40.3%。HepG2 細胞添加 EGb761 (1000 μg/mL)其 p53 蛋白質與 PCNA 之表現量分別爲控制組之 173.7%、85.1%。體內實驗部分，將大白鼠(Fischer 344; F344) 隨機分成四組：控制組(無肝癌無添加 EGb761)、無肝癌添加 EGb761 組、肝癌無添加 EGb761 組及肝癌添加 EGb761 組。肝癌老鼠以二乙亞硝胺(diethylnitrosamine; DEN)－氟乙醯胺(2-acetylaminofluorene; 2-AAF)－手術切除肝臟左大葉(爲期五星期)之三階段模式誘發老鼠肝癌形成。之後於添加 EGb761 組之老鼠飼料中額外添加 EGb761 (50 毫克/公斤體重/天)。給予 EGb761 四星期後觀察肝臟之病理切片以評估肝癌細胞之增殖情形。於體內實驗結果顯示：老鼠之肝重、實驗期攝食量於各組間皆無顯著差異，控制組之體重增加量顯著較肝癌組爲高($p < 0.05$)。於肝臟之病理切片發現，肝臟癌結之最大面積與數目於肝癌添加 EGb761 組與肝癌無添加 EGb761 組間並無顯著差異。於本研究體外實驗中發現 EGb761 可明顯抑制人類肝癌細胞株(HepG2 與 Hep3B2.1-7)之增殖情形並顯著降低其存活率，且發現 EGb761 能提高肝癌細胞株(HepG2)之抑癌蛋白 p53 蛋白質並降低 PCNA 之表現量。於體內實驗中發現肝癌之誘發造成老鼠之體重增加量顯著降低，於病理切片發現添加 EGb761 (50 毫克/公斤體重/天)四星期後，對於肝癌並無顯著之改善。

英文摘要

The purpose of this study was to investigate the effects of EGb761 containing 22-27% ginkgo-flavone glycosides and 5-7% terpenoids on hepatocelluarlar carcinoma. In the in vitro experiment, EGb761 was added by different concentrations (0-1000 μg/mL) in the medium of human hepatocelluarlar carcinoma (HCC) cell lines (HepG2 and

Hep3B2.1-7) for 24 to 48 hours. The effects of EGb761 on cell proliferation and lactate dehydrogenase (LDH) leakage in HepG2 and Hep3B2.1-7 cells and the expression of p53 and proliferating cell nuclear antigen (PCNA) in HepG2 cells were determined. The results showed that EGb761 (50-1000 μg/mL) significantly suppressed cell proliferation and increased LDH leakage of HepG2 and Hep3B2.1-7 cells ($p < 0.05$). Cell proliferation of HepG2 and Hep3B2.1-7cells treated with EGb761 (1000 μg/mL) was 45.3% and 38.8% of the control ($p < 0.05$), respectively. LDH leakage of HepG2 cells without EGb761 and with EGb761 (1000 μg/mL) was 6.7% and 37.7%, respectively, and that of Hep3B2.1-7cells without EGb761 and with EGb761 (1000 μg/mL) was 7.2% and 40.3%, respectively. The expression of p53 and PCNA proteins in HepG2 cells treated with EGb761 (1000 μg/mL) was 173.7% and 85.1%, respectively. In the in vivo experiment, Fischer 344 (F344) rats were randomly divided into four groups: control (no HCC without EGb761), no HCC with EGb761, HCC without EGb761, and HCC with EGb761 groups. HCC rats were induced by the 3-stage model of diethylnitrosamine (DEN)—2-acetylaminofluorene (2-AAF)—partial hepatectomy (a left-leaf liver resection) for five weeks. After HCC induction, rats were fed with EGb761 (50 mg/kg body weight/day) for four weeks to grossly evaluate cell proliferation by pathological assessment. The results showed that liver weight and food intake during the experimental period did not differ among the groups. Body weight gain of the control group was significantly higher than that of the HCC groups ($p < 0.05$). The maximal area and number of nodules did not significantly differ between the HCC groups with and without EGb761. In conclusion, EGb761 significantly suppressed proliferation and reduced viability of HepG2 and Hep3B2.1-7 cells, increased p53 expression and decreased PCNA expression in HepG2 cell in vitro. The in vivo experiment showed that rats with HCC had significantly lower weight gain. The pathological assessment showed that EGb761 treatment (50 mg/kg body weight/day) for 4 weeks did not obviously improve HCC.