• 研究快报 BRIEF REPORT •

银杏叶类黄酮对人胃癌细胞 BGC823 体外的增殖抑制作用

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收稿日期: 2005-06-28 接受日期: 2005-07-16

Inhibitory effects of *Ginkgo biloba* leaf flavonoids on proliferation of human gastric cancer cell line BGC823 *in vitro*

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Received: 2005-06-28 Accepted: 2005-07-16

Abstract

AIM: To extract the flavonoids from *Ginkgo biloba* leaf, and to investigate its inhibitroy effects on the proliferation of human gastric cancer cell line BGC823 cultured *in vitro*.

METHODS: Ethanol (700 mL/L) was used to extract the flavonoids from the leaf of *Ginkgo biloba*. Three wavelength spectrophotometry was used to determine the content of flavonoids in the extracts. Human gastric cancer cells BGC823 cultured *in vitro* were treated with different concentrations of the flavonoids, and then the proliferation of the cells was detected by MTT assay and flow cytometry.

RESULTS: The content of flavonoids in the extracts was 140 mg/g. The flavonoids from *Ginkgo biloba* leaf inhibited the proliferation of BGC823 cells in a dose-dependent manner. The rate of cells in S phase was notably increased as compared with that in the controls (42.17±0.50% *vs* 32.13±0.45%, $P = 0.001$), and the apoptotic rate of the cells was also increased (4.10±0.03% *vs* 2.21±0.01%, $P = 0.002$).

CONCLUSION: *Ginkgo biloba* leaf flavonoids can inhibit the proliferation of human gastric cancer cell line BGC823 by affecting the cycle the cells.

Key Words: *Ginkgo biloba* leaf flavonoids; Gastric cancer

Zhang F, Yang GW, Zhang JF, An LG. Inhibitory effects of *Ginkgo biloba* leaf flavonoids on proliferation of human gastric cancer cell line BGC823 *in vitro*. Shijie Huaren Xiaohua Zazhi 2005;13(21):2627-2629

摘要

目的: 提取并测定银杏叶类黄酮(芦丁)的含量; 探讨银杏叶类黄酮对体外培养的人胃癌细胞BGC823的增殖抑制作用.

方法: 乙醇法(700 mL/L)提取银杏叶类黄酮; 三波长分光光度法测定提取物中类黄酮(芦丁)的含量; MTT法及流式细胞技术观察提取物对人胃癌细胞BGC823的增殖抑制作用.

结果: 提取物中类黄酮(芦丁)含量为140 mg/g. MTT法证实银杏叶类黄酮对人胃癌细胞BGC823增殖有抑制作用, 且呈剂量依赖效应. 流式细胞术分析表明银杏叶类黄酮将胃癌细胞BGC823的生长周期阻滞于S期, 与对照组相比明显增加(42.17±0.50% *vs* 32.13±0.45%, $P = 0.001$), 凋亡细胞数与对照组相比也显著增加(4.10±0.03% *vs* 2.21±0.01%, $P = 0.002$).

结论: 银杏叶类黄酮对人胃癌细胞BGC823增殖有抑制作用, 并能阻抑细胞周期进程, 诱导细胞凋亡.

关键词: 银杏叶类黄酮; 胃癌

张凤, 杨桂文, 张金凤, 安利国. 银杏叶类黄酮对人胃癌细胞BGC823体外的增殖抑制作用. 世界华人消化杂志 2005;13(21):2627-2629
http://www.wjgnet.com/1009-3079/13/2627.asp

0 引言

银杏(Ginkgo biloba L.)又名白果树, 银杏叶提取物(GBE)主要药用成分之一为类黄酮[1-3], 用于对心血管、动脉硬化、高血压等疾病的治疗, 具有独特药理活性和巨大的临床应用价值[4-9]. Lee *et al*[10]报道银杏提取物中的双黄酮—银杏黄素或异银杏黄素10 μmol/L能抑制ConA或LPS诱导的淋巴细胞增殖, Chao *et al*[11]研究表明, 银杏提取物EGb761能够抑制肝癌细胞HepG2和Hep3B的增殖, Kim *et al*[12]研究显示, 银杏提取物EGb761能够激活caspase-3诱导口腔牙槽癌细胞凋亡. 据此我们推测银杏叶提取物也许能够通过抑制细胞增殖从而抑制胃癌细胞的生长, 在胃癌治疗中发挥有益作用. 我们研究银杏叶提

取物中类黄酮含量并探讨其对胃癌细胞BGC823细胞增殖的抑制作用, 为进一步开发银杏这一我国特有植物物种资源的应用价值提供基础资料.

1 材料和方法

1.1 材料 银杏叶; BGC823细胞株; RPMI-1640培养基(Gibco); 小牛血清(杭州四季清公司); 二甲基亚砜(DMSO); 噻唑蓝(MTT)(Sigma); 芦丁标准品(中国药品检验所). 类黄酮的提取(700 mL/L乙醇法)参照秦雪莲*et al*[13-15]的方法, 称取银杏叶干粉10 g, 加700 mL/L乙醇400 ml, 混匀, 40℃超声振荡50 min, 8 000 *g*离心20 min, 收集上清. 滤渣再加700 mL/L乙醇300 mL, 混匀, 40℃超声振荡30 min, 8 000 *g*离心20 min, 收集上清. 滤渣再加700 mL/L乙醇100 mL, 混匀, 40℃超声振荡20 min, 8 000 *g*离心20 min, 收集上清. 将所得的3次上清液合并, 减压浓缩并将浓缩液置于硫酸纸上, 40℃干燥箱中干燥, 所得干燥品即为银杏叶粗黄酮. 称取芦丁标准品5 mg溶于水中并定容于100 mL容量瓶中, 即为芦丁母液; 取母液0.5, 1.0,1.5,2.0, 2.5, 3.0, 3.5 mL于容量瓶中, 每一容量瓶用水加至3.5 mL, 再用10 g/L $AlCl_3$定容至5 mL; 测定λ_1 = 470 nm, λ_2 = 420 nm, λ_3 = 370 nm三波长处的吸光度A值. 按公式$\triangle A=A_2-\{[(\lambda_2-\lambda_3)A_1+(\lambda_1-\lambda_2)A_3]/(\lambda_1-\lambda_3)\}$计算$\triangle A$, 根据$\triangle A$与浓度关系绘制标准曲线. 样品中类黄酮含量的测定: 称取银杏叶粗黄酮5 mg溶于水中, 并定容于5 mL容量瓶中, 即为粗黄酮溶液, 取此液2.5 mL于5 mL容量瓶中, 用水加至3.5 mL, 用10 g/L $AlCl_3$定容至刻度, 测定λ_1 = 470 nm, λ_2 = 420 nm, λ_3 = 370 nm三波长处的吸光度A值, 计算样品中黄酮含量. 10 g银杏叶中提取得到类黄酮1.95 g, 得率为19.5%. 根据$\triangle A$与浓度关系绘制标准曲线, 回归方程为: Y=31.096x+0.013 4, R^2=0.999 7. 样品中类黄酮含量计算可知1 mg提取物中含黄酮140 μg, 进而根据提取物得率计算银杏叶中类黄酮含量为2.73%.

1.2 方法 取对数生长期的胃癌细胞BGC823细胞以5×10^7/L浓度接种于96孔培养板, 每孔100 μL, 37℃, 50 mL/L CO_2培养箱中培养. 24 h后分别接入不同浓度的无菌的银杏叶类黄酮溶液100 μL, 使最终浓度分别为60, 80, 100, 150, 200, 250 g/L, 各组设8个重复孔, 对照组加100 μL培养液, 在37℃, 50 mL/L CO_2条件下继续培养48 h. 实验终止前加入新配制的5 g/L的MTT溶液20 μL, 混匀, 再继续培养4 h. 弃去上清, 每孔加DMSO 200 μL, 充分振荡30 min, 溶解MTT沉淀物, 490 nm测定每孔的吸光度A值[16-19]. 计算抑制率: 抑制率=(1-实验组A值/对照组A值)×100%. 另将5×10^7/L细胞接种于2个6 cm培养皿中, 24 h后加入无菌银杏叶类黄酮溶液, 使其终浓度为150 g/L, 对照组加等量的培养液.继续培养48 h, 终止培养后制成单细胞悬液, 调整细胞浓度为1×10^9/L, 离心, 用同体积700 mL/L的冷乙醇固定1 h, 离心, PBS洗涤2次, 将细胞悬液和碘化丙啶(PI)等体积混合, 4℃放置30 min, 放入流式细胞仪样品室, 488 nm检测细胞周期和细胞凋亡的情况[20,21].

统计学处理 用SPSS 10.0统计学软件和t检验处理数据, 所有数据均用mean±SD表示.

2 结果

2.1 银杏叶类黄酮对胃癌细胞BGC823的抑制作用 银杏叶类黄酮对肿瘤细胞BGC823增殖有抑制作用, 并呈现明显的剂量依赖效应 (表1).

表1 银杏叶类黄酮对胃癌细胞增殖的抑制作用

黄酮(mg/L)	吸光度(A)	抑制率(%)
0	0.675 ± 0.096	–
60	0.596 ± 0.084[b]	11.5 ± 1.4
80	0.555 ± 0.164[a]	17.4 ± 6.6
100	0.484 ± 0.057[b]	28.1 ± 2.4
150	0.361 ± 0.094[b]	46.4 ± 2.8
200	0.168 ± 0.022[b]	75.2 ± 0.5
250	0.096 ± 0.013[b]	85.9 ± 0.4

[a]P<0.05, [b]P<0.01 *vs* 0 mg/L.

2.2 流式细胞仪分析结果 银杏叶类黄酮使胃癌细胞BGC823的细胞周期滞留于S期, 并且能够诱导细胞凋亡(表2).

表2 流式细胞仪分析的BGC823细胞周期分布 (mean ± SD %)

银杏叶黄酮类	G_0–G_1期	S期	G_2–M期	凋亡率
对照	58.45 ± 0.35	32.13 ± 0.45	9.41 ± 0.14	2.21 ± 0.11
150 mg/L	57.72 ± 0.45	42.17 ± 0.50[b]	0.11 ± 0.08[b]	4.10 ± 0.03[b]

[b]P<0.01 *vs* 对照.

3 讨论

在类黄酮化合物的紫外光谱吸收中, 主要吸收带是由304-350 nm的吸收带I和240-280 nm的吸收带II组成. 因为银杏叶类黄酮提取物中其它成分在带I和带II范围内也有一定程度的吸收, 会对类黄酮的测定产生干扰. 加入铝盐使类黄酮与铝离子形成稳定的配合物, 吸收带I会明显红移, 同时吸光度也大大增加. 因此, 选择铝配合物显色体系来测定总黄酮的含量可以去除其他成分的干扰[22,23], 同时选择三波长—光谱法能够有效地消除吸收峰不对称给定量分析造成的影响, 提高定量分析的准确度. 通过数据分析可知银杏叶粗提取物中类黄酮含量为140 mg/g, 银杏叶中类黄酮的含量约为2.73%.

通过MTT法观察银杏叶类黄酮对人胃癌细胞增殖的影响表明, 银杏叶类黄酮对人胃癌细胞的增殖有抑制作用, 而且存在明显的剂量依赖效应. 我们在中效浓度附近选择了150 mg/L进一步用流式细胞仪分析其对胃癌细胞BGC823的作用, 结果表明处理的胃癌细胞BGC823 S期细

胞比例明显增加(42.17±0.50 *vs* 32.13±0.45, $P = 0.001$), 并且能够诱导细胞凋亡(4.10±0.03 *vs* 2.21±0.01, $P = 0.002$), 这说明银杏叶类黄酮能够把胃癌细胞BGC823的细胞周期阻滞在S期, 阻碍其向G_2/M期的转换, 减缓其分裂的速度, 从而抑制其增殖并诱导细胞凋亡. 这与许多研究者对抗癌活性物质研究的实验结果类似[24-28], 其诱导癌细胞凋亡的机制可能与p53和Fas有关[29,30]. 然而, 银杏叶类黄酮如何通过阻抑细胞周期进程并诱导细胞凋亡起到抗肿瘤作用的至今还不清楚. 因此, 对于银杏叶类黄酮抗肿瘤的作用机制尚需进一步研究.

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