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水飞蓟宾对猪脑基底动脉 5-脂氧酶活性的抑制作用[1]

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Inhibitory effect of silybin on the activity of 5-lipoxygenase of the porcine cerebral basilar artery

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ABSTRACT The chopped porcine cerebral basilar arteries (PCBA) were incubated in the modifed Tyrode solution with calcium ionophore calcimycin (A-23187, Cal) 10 μmol/L in the presence of arachidonic acid 30.6 μmol/L and indomethacin, a cyclooxygenase inhibitor 2.8 μmol/L. The culture was extracted and purified with a SEP-PAK column (SEP-PAK C_{18} Cartridge, Waters). The bioassay of the extract was then made on the isolated guinea pig ileum with the standard leukotriene D_4 (LTD_4) 200 pg/ml as a reference. The acetylcholine, histamine and 5-HT released by the ileum was preblocked by atropine 1 μmol/L, diphenhydramine 1 μmol/L and cyproheptadine 3 nmol/L. The contraction produced by the extract on the ileum showed the same characterestic as LTD_4, and was blocked by the specific LTs antagonist FPL 55712 100 ng/ml. Reversed phase high performance liquid chromatography analysis indicated the presence of peaks co-chromatographing with standard LTB_4, C_4 and D_4. The retention times of LTB_4, C_4 and D_4 in our system were 2.2, 3.2, 5.5 min respectively. The eluates of peaks co-chromatographing with LTB_4, C_4 and D_4 were collected and tested for contractile activity on the guinea pig iluem. Only the substances which had the similar LTC_4 and LTD_4 retention times exhibited contractile activities. Hence we concluded that the substances of LTs which had biological activities

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were mainly LTC_4 and D_4. The amount of LTs released from PCBA was 70 ± 15 pg/100 mg tissue. When PCBA was preincubated with silybin 100 and 500 μmol/L, the amounts of LTs released were 27 ± 12 and 14±6 pg/100 mg tissue, respectively ($P<0.01$). This result suggests that silybin can inhibit the activity of 5-lipoxygenase of cerebral blood vessel and may protect the brain from ischemia.

KEY WORDS silymarin; cerebral arteries; lipoxygenases; leukotrienes; biological assay; high pressure liquid chromatography; SRS-A

提要 离体猪脑基底动脉与吲哚美辛,AA 和 calcimycin 一起培养能产生具高度生物活性的物质·经生物鉴定及反相 HPLC 测定证明为白三烯 (leukotrienes, LTs),其生物活性部分主要是 LTC_4, D_4. 释放量为 70±15 pg/100 mg. 加入 silybin 0.1, 0.5 mmol/L 后, LTs 的释放显著减少, 分别为 27±12, 14±6 pg/100 mg ($P<0.01$). 提示 silybin 能抑制脑血管 5-脂氧酶活性, 可能对脑缺血具保护作用.

关键词 水飞蓟宾; 脑动脉; 脂氧化酶; 白细胞三烯; 生物鉴定; 高压液相色谱法; 慢反应物质 A

黄酮类及有关化合物对花生四烯酸 (arachidonic acid, AA)的环氧酶和脂氧酶代谢途径有不同的抑制作用. 水飞蓟宾(silybin, Sil)抑制大豆脂氧酶的 IC_{50} 为 150 μmol/L. 对大鼠肺、脾脂氧酶也有抑制作用, 对环氧酶抑制作用较弱[1]. 有证据表明 Sil 对肝脏的保护作用与其抑制细胞膜脂质的过氧化有关[2].

脑组织和脑血管含有丰富的脂氧酶, 具有合成白三烯(LTs)的能力[3], 脑缺血时 LTs 释放增加. 文献报道, 槲皮素(quercetin, Quer)是较强的脂氧酶抑制剂, 在脑组织中浓度达 10 μmol/L 时可抑制 LTs 的释放[4]. Quer 的结构类似物 Sil 对脑血管脂氧酶的作用尚未见文献报道. 本文以 Quer 为对照研究 Sil 对培养的猪脑基底动脉合成及释放 LTs 的影响, 为防治脑血管疾病的应用提供一定的实验依据.

MATERIALS AND METHODS

标准品 LTB_4, C_4, D_4 系 Merck Frosst Canada 公司惠赠. 水飞蓟宾由本院药厂提供(96%; mp 167-8℃) Quer、阿托品、苯海拉明由北京药品生物制品检定所生产,赛庚啶(cyproheptadine)、卡西霉素(calcimycin, A-23187)、AA(99%纯) 均系 Sigma 产品. FPL-55712 由 Naso Whirl-Pak 公司生产. 吲哚美辛 (indomethacin)由上海第十七制药厂生产.

所用水飞蓟宾制成葡甲胺盐[5], 用 4% 聚乙烯吡咯烷酮(PVP)配成溶液,用 HCl 1 mol/L 调 pH 至 7-8. Quer 在临加药前用 10% $NaHCO_3$ 溶液(pH 8) 配制. Calcimycin 用无水乙醇配制, 最终含醇量不超过 0.05%, 其它均用盐水配制.

离体猪脑基底动脉血管的培养[6] 取新断头的猪脑基底动脉,置于冰冷的 Tyrode 液中保存. 除去血管周围的结缔组织和脑组织,称重,剪碎, 加入 Tyrode 液.

Quer, Sil 按终浓度分别为 20, 100, 500 μmol/L 加入各管, 于 37℃ 振荡培养 20 min, 再依次加入吲哚美辛 2.8 μmol/L, AA 30.6 μmol/L, calcimycin 10 μmol/L 培养 60 min, 培养结束后, 迅速置冰浴中止反应. 溶剂对照组所用量与用药组相同.

LTs 的提取[7] 上述冷却的培养液用 HCl 1 mol/L 酸化, pH 约为 5.4. 而后用依次通过 H_2O,无水乙醇,H_2O 各 10 ml 预处理过的 SEP-PAK 柱(SEP-PAK C_{18} Cartridge, Waters) 3 次, 再用 H_2O 5 ml, 石油醚 5 ml 洗去杂质. 最后用甲酸甲酯 2 ml 洗脱 LTs[8], 收集甲酸甲酯部分, 在 N_2 气流下吹干, -40℃ 保存. 一部分供反相高压液相 (HPLC) 定性, 一部分供生物鉴定.

生物鉴定[9] 豚鼠, ♂,体重 276±SD 22 g. 击昏, 剪取回肠, 冲洗内容物, 制成长约 1-2 cm 的肠段, 置于 Tyrode 液(Ca^{2+} 离子量减至 1/4)10 ml 37℃ 恒温浴池中, 通 95%O_2+

5%CO_2，加 2 g 负荷作为静息张力，经机械电换能器，用 LM 14-204 型自动平衡记录仪描记张力变化曲线。肠段平衡至少 30 min，待收缩稳定后，加入阿托品 1 μmol/L，苯海拉明 1 μmol/L 和赛庚啶 3nmol/L。稳定 5 min，加入上述 LTs 提取物（用 Tyrode 液溶解）或标准品 LTD_4 200 pg/ml，观察对回肠张力的影响。提取物中所含 LTs 的量由下述标准曲线方程计算。

标准曲线方程的建立 标准品 LTD_4 溶于无水乙醇配成标准液，使浓度为 10 ng/ml。

用上述生物鉴定方法，按标准品 LTD_4 的终浓度分别为 0.1，0.2，0.3，0.4 ng/ml 加入，观察并记录回肠段的收缩曲线。以标准品 LTD_4 浓度对回肠张力的增加量回归得标准曲线方程：$Y = 0.2245 + 1.207 \times 10^{-3}X$　$r = 0.9989$。

反相HPLC[10] 采用 Waters 510/590 型梯度 HPLC 仪，层析柱为 C_{18} μ-Bondapak 柱（0.39 × 30 cm，流动相为乙腈：水：三氟乙酸 70:30:0.0008 行线性洗脱。于 20 min 内使三氟乙酸浓度从 0.0008% 增至 0.02%。流速为 2 ml/min。采用 Waters 490 型程控多波长检测器，检测波长为 280 nm。

样品中 LTB_4，C_4，D_4 根据标准品 LTB_4，C_4，D_4 的保留时间而定性。收集样品在 280 nm 有较大吸收时的流出液，N_2 气流下吹尽乙腈，剩下的水溶液冰冻干燥。-40℃ 保存，进行生物鉴定。

RESULTS

离体猪脑基底动脉在吲哚美辛 2.8 μmol/L 存在时，与 AA 30.6 μmol/L，Ca1 10 μmol/L 一起培养后，产生并释放出大量的生物活性物质。豚鼠回肠用阿托品、苯海拉明、赛庚啶分别阻断培养时所释放的乙酰胆碱、组胺、5-羟色胺对其张力的影响后，再加入提取物，回肠张力明显增加，且与标准品 LTD_4 引起的回肠收缩颇为相似，1-2 min 潜伏期后，开始收缩，持续 2-3 min。如果先加入 LTs 受体拮抗剂 FPL 55712 100 ng/ml，再加入提取物，回肠张力无变化。如果先加提取物，待回肠收缩达最高峰时加入 FPL-55712 100 ng/ml，回肠的收缩迅速被拮抗（Fig 1）。说明提取物中含 LTs。

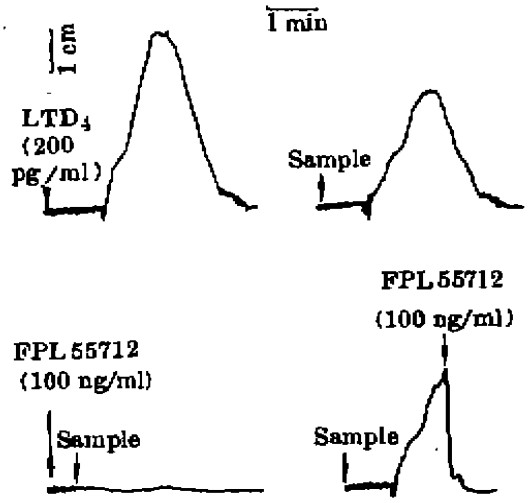

Fig 1. Contractions of strips of guinea pig ileum to leukotriene D_4 and to the extract eluted from SEP-PAK C_{18} Cartridge. The ileum was preblocked by atropine 1 μmol/L, diphenhydramine 1 μmol/L and cyproheptadine 3 nmol/L.

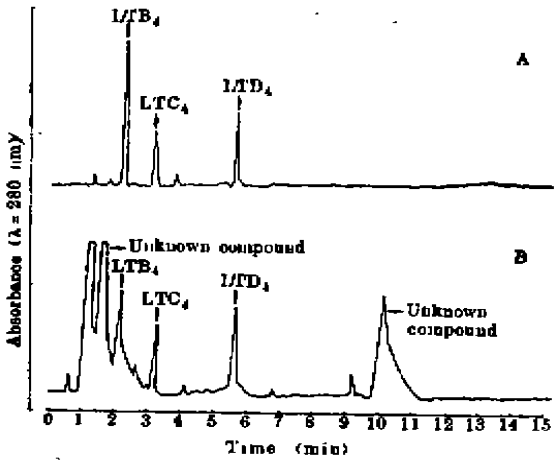

Fig 2. Reversed-phase HPLC of the standard leukotrienes B_4, C_4, D_4 (A) and the sample (B) using a gradient mobile phase. The gradient between 0.0008% (0.1 mmol/L) trifluoroacetic acid (TFA) and 0.02% (2.5 mmol/L) TFA over 20 min (70% acetonitrile in water) was used to separate leukotrienes B_4, C_4, and D_4. The flow rate was 2 ml/min.

Quer 和 Sil 20, 100, 500 μmol/L 均能抑制猪脑基底动脉合成并释放 LTs, 溶剂组无影响. Quer 和 Sil 与所释放的 LTs 量之间存在着较好的量-效关系(Tab 1).

Tab 1. Inhibitory effects of quercetin and silybin on the activity of 5-lipoxygenase of the isolated porcine cerebral basilar arteries. n = number experiments, $\bar{x} \pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$.

Drug (μmol/L)	n	Release of leukotrienes (pg/100 mg tissue) Quercetin	n	Silybin
Control	7	70±19	6	70±15
20	5	14±17**	6	65±20*
100	5	8±18***	6	27±12***
500	6	0***	6	14±6***

提取物经反相 HPLC 及对其收集物的生物鉴定分析,进一步证明其中含 LTB_4, C_4, D_4. Fig 2 A 为标准品 LTB_4, C_4, D_4 的 HPLC图谱, Fig 2 B 为样品的 HPLC 图谱. 各 LTs 类物质 LTB_4, C_4, D_4 的保留时间分别为 2.2, 3.2 和 5.5 min (Fig 2).

HPLC 收集液经生物鉴定表明, 在 LTC_4, D_4 保留时间相近处的流分中所含物质具有生物活性, 且其引起的回肠收缩可被 FPL-55712 100 ng/ml 所拮抗,表明生物鉴定中引起回肠收缩的主要成分为 LTC_4, D_4.

生物鉴定结果还表明, 当不加入外源性的 AA 时, 几无 LTs 的产生及释放 ($n = 3$).

DISCUSSION

本实验结果表明, Quer 20, 100, 500 μmol/L 能非常显著地抑制猪脑基底动脉合成及释放 LTs,与文献报道相符,说明本文方法是可靠的. 其结构类似物 Sil 100, 500 μmol/L 也能非常显著地抑制猪脑基底动脉 5-脂氧酶的活性, 从而使 LTs 的合成及释放减少.

猪脑基底动脉血管壁中存在 5-脂氧酶系统, 经 Cal (或致敏后抗原)攻击后, 可释放出 LTs,但量不多, 在本实验中加入环氧酶抑制剂吲哚美辛, 抑制了环氧酶代谢途径, 使更多的 AA 通过脂氧酶代谢, 从而使释放的 LTs 增加[11].

当不加入外源性的 AA 时, 几乎没有 LTs 的释放, 说明正常情况下, 脑血管合成并释放的 LTs 量极少, 但脑血管具有强的合成 LTs 的能力, 加入外源性 AA 后, LTs 释放量大大增加.

脑缺血时脑组织及脑血管中游离的 AA 明显增加[12], 其 5-脂氧酶代谢物 LTs 的量也增加. Sil 可抑制脑血管5-脂氧酶合成及释放 LTs, 提示 Sil 可能对脑缺血具有保护作用.

采用生物鉴定测定样本中 LTs 的含量是很敏感的方法, 其检出量约为 0.1 ng/ml, 而 HPLC 需要 10 ng/ml. 借助标准品可用生物鉴定法进行定量, HPLC 法可确定提取物中所含成分.

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高钾饮食对肾血管高血压大鼠血压、前列腺素、尿激肽释放酶的影响

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Effects of dietary K on blood pressure, prostaglandin, and kallikrein in renovascular hypertensive rats

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ABSTRACT The effects of dietary K (food and tap water both containing 1% KCl) on blood pressure and renal prostaglandin-kallikrein-kinin system were investigated in Wistar rats made hypertensive by constriction of left renal artery. Dietary K attenuated the development of hypertension and increased urine volume accompanied by increased excretion of K, but by uninfluenced excretion of Na. Dietary K also increased the urinary excretion of kallikrein, PGE_2 and aldosterone in Goldblatt hypertensive rats. There was no significant difference in the values of plasma Na between the two groups with and without dietary K. These results suggest that dietary K may attenuate the development of hypertension, increase urine volume via the mechanism of enhancing production of renal PGE_2 and kallikrein in hypertensive rats.

KEY WORDS dietary potassium; blood pressure; aldosterone; prostaglandins; kallikrein; renovascular hypertension; diuresis

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提要 放射免疫法(RIA)测定前列腺素 E_2(PGE_2)，分光光度法测定尿激肽释放酶，观察到高钾饮食可显