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哒唑氧苯对离体猪脑基底动脉花生四烯酸代谢的影响[1]

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Effect of dazoxiben on the metabolism of arachidonic acid in isolated porcine basilar arteries[1]

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ABSTRACT Dazoxiben, a selective TXA_2 synthetase inhibitor, was studied in the incubating sections of porcine basilar arteries with arachidonic acid (AA) 50 μmol / L and calcimycin (calcium inophore A-23187) 50 μmol / L. TXB_2 and 6-keto-$PGF_{1\alpha}$ were determined by radioimmunoassay. Leukotrienes (LT) were extracted and purified with SEP-PAK column, identified by HPLC and determined by bioassay with ileum of guinea pig. The results showed that the production of TXB_2 was unaltered whether or not the incubation of arteries were induced by AA or calcimycin. Dazoxiben and indomethacin 0.05-50 μmol / L had no effects on the production of TXB_2. However, dazoxiben 0.5, 5 and 50 μmol / L increased the production of 6-keto-$PGF_{1\alpha}$ by 16.3%, 19.0% and 30.7%, respectively. Indomethacin 0.5, 5 and 50 μmol / L decreased the production of 6-keto-$PGF_{1\alpha}$ by 22.3%, 24.9% and 24.0%, respectively. Meanwhile dazoxiben 1, 10 and 100 μmol / L decreased the production of LT by 33.4%, 45.6% and 66.4%, respectively. These results suggest that the protective effect of dazoxiben on the damages which resulted from brain ischemia may be related to the change of TAX_2 / PGI_2 balance in the brain tissue as well as the inhibition of production of LT.

KEY WORDS dazoxiben; indomethacin; prostaglandins X; thromboxane A_2; basilar artery; leukotrienes; radioimmunoassay

提要 哒唑氧苯和吲哚美辛 0.05-50 μmol / L 对离体猪脑基底动脉产生 TXA_2 无影响. 哒唑氧苯 0.5-50 μmol / L 能显著增加 PGI_2 的产生, 而相同浓度的吲哚美辛显著抑制 PGI_2 的产生. 同时哒唑氧苯 1-100 μmol / L 能显著抑制 LT 的产生. 结果进一步阐明了哒唑氧苯对脑缺血保护作用的机理.

关键词 哒唑氧苯; 吲哚美辛; 前列腺素 X 类; 血栓素 A_2; 基底动脉; 白三烯; 放射免疫测定

哒唑氧苯(dazoxiben, UK-37248)是一种选择性血栓素 A_2(thromboxane A_2, TXA_2)合成酶抑制剂, 能够抑制血小板聚集[1,2]. 作者发现[3]哒唑氧苯能够显著降低麻醉兔血浆 TXA_2 水平, 升高前列环素(prostacylin, PGI_2)水平, 对正常脑血管阻力无影响, 但可对抗椎动脉注射花生四烯酸(arachidonic acid, AA)引起的脑血管阻力增加, 保护脑缺血引起的损害. 而吲哚美辛(indomethacin)能够显著降低兔血浆 TXA_2, PGI_2 水平, 同时增加兔正常脑血管阻力, 不能对抗由椎动脉注射 AA 引起的脑血管阻力的增加. 本文用培养离体猪脑基底动脉的方法, 观察哒唑氧苯对 AA 两条代谢途径的影响, 进一步探讨其保护脑缺血的作用机理.

MATERIALS AND METHODS

哒唑氧苯(本院有机化学教研室合成); AA-Na (99%), 卡西霉素(calcimycin, A-23187, Sigma); 吲哚美辛(上海十七制药厂): TXB_2, 6-keto-$PGF_{1\alpha}$ 放射免疫测定盒(北京军区总医院); LTC_4, D_4(Merck Frosst Canada 公司惠赠).

离体猪脑基底动脉培养 取新鲜猪脑基底动脉, 置充有 95% O_2 + 5% CO_2 的冷 krebs 液中, 除去周围结缔组织, 吸干水分, 称重, 剪碎, 加入 2 ml Krebs 液. 置 37℃ 水浴孵育 10

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min, 然后, 加呋唑氧苯和吲哚美辛, 不加药物者作为空白对照组. 孵育 20 min, 加入 AA-Na 50 μmol/L, calcimycin 50 μmol/L, 继续孵育 60 min, 冰冻中止反应, 离心取上清液, 待测药物对环氧酶代谢途径的影响. 观察呋唑氧苯对白三烯类(leukotrienes, LT)代谢的影响时, 培养基底动脉应加入吲哚美辛 2.8 μmol/L, 以阻断 AA 环氧酶代谢途径, 使 LT 合成增加[4].

TXB_2, 6-keto-$PGF_{1\alpha}$ 的提取与测定 按文献[5]将上述培养液用 HCl 1 mol/L 调节 pH 3.5-4, 用重蒸乙酸乙酯提取 2 次, 合并两次提取液, N_2 吹干, 置-40℃贮存. 用放射免疫法测定 TXA_2 和 PGI_2 稳定的代谢产物 TXB_2 和 6-keto-$PGF_{1\alpha}$.

LT 的提取与鉴定 按文献[6]采用 SEP-PAK 柱 (SEP-PAK C_{18} Cartridge, Waters) 提取分离培养液中 LT. 首先顺次用 10 ml 水、10 ml 无水乙醇、10 ml 水洗涤处理 SEP-PAK 柱, 然后将培养液用 HCl 1 mol/L 调 pH 约为 5.4 后通过柱三次, 再用水 5 ml, 石油醚 5 ml 洗去杂质, 最后用 2 ml 甲酸甲酯洗脱 LT, N_2 吹干, 置-40℃备用. 采用反相 HPLC 法定性鉴定提取液中 LT[7], 用 Waters 510/590 HPLC 仪, 层析柱为 $C_{18}\mu$-Bondpak 柱(0.39 × 30 cm), 流动相为乙腈:水:三氟乙酸(0.0008%-0.02%), 进行线性洗脱, 检测波长为 280 nm. 采用豚鼠回肠生物检定法[8]测定提取液中 LT 含量, 描记提取液对回肠的收缩张力, 从 LTD_4 标准品对回肠收缩张力所做的标准曲线上计算出样品中 LT 的量. 标准曲线方程为 $Y = 0.2245 + 0.001207\ X$, $r = 0.9989$.

RESULTS

药物对离体猪脑基底动脉产生 TXB_2, 6-keto-$PGF_{1\alpha}$ 的影响 结果见 Tab 1. 呋唑氧苯和吲哚美辛 0.05-50μmol/L 对离体猪脑基底动脉产生 TXB_2 无影响, 而呋唑氧苯 0.5, 5 和 50 μmol/L 能显著增加基底动脉产生 6-keto-$PGF_{1\alpha}$ 的量, 比对照组分别增加 16.3%, 19.0% 和 30.7%, 有一定的量-效关系.相同浓度的吲哚美辛可显著抑制基底动脉产生 6-keto-$PGF_{1\alpha}$, 与对照组相比, 分别抑制 22.3%, 24.9% 和 24.0%. 实验还表明, 不管有无外源性 AA 或 calcimycin 刺激, 猪脑基底动脉产生的 TXB_2 量无显著差异, 分别为 16 ± 4 ng/100 mg 组织 ($n=8$, 无 AA, calcimycin 刺激)和 17 ± 4 ng/100 mg 组织 ($n=16$, 有 AA, calcimycin 刺激)

Tab 1. Effects of dazoxiben, indomethacin on production of thromboxane B_2(TXB_2) and 6-keto-prostaglandin $F_{1\alpha}$(6-keto-$PGF_{1\alpha}$) induced by arachidonic acid, calcimycin (A-23187) in porcine basilar arteries. $\bar{x} \pm SD$ (ng/100 mg tissue). $^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$.

Drug(μmol/L)	n	TXB_2	n	6-keto-$PGF_{1\alpha}$
Control	16	17±4	11	86±13
Dazoxiben				
0.05	12	15±4*	11	92±13*
0.5	14	15±3*	12	100±12**
5	15	16±4*	11	102±20**
50	15	15±4*	11	112±24***
Indomethacin				
0.05	14	15±4*	9	79±15*
0.5	12	17±3*	8	67±18**
5	9	15±3*	9	65±14***
50	12	15±5*	6	65±13***

药物对离体猪脑基底动脉产生 LY 的影响 结果见 Tab 2. 对照组培养液经反相 HPLC 定性测定表明含有 LTB_4、D_4 及 D_4. 豚鼠回肠生物检定表明相当于标准品 LTC_4, D_4 保留时间的流出部分可引起豚鼠回肠收缩, 而且此收缩可被 LT 受体拮抗剂 FPL 55712 所阻断, 提示提取物中主要生物活性部分为 LTC_4 及 D_4. 呋唑氧苯 1, 10 和 100 μmol/L 能显著抑制 LT 的产生, 与对照组相比, 分别抑制 33.4%, 45.6% 和 66.4%, 呈较好的量-效关系.

Tab 2. Effects of dazoxiben on the production of leukotrienes induced by arachidonic acid, calcimycin (A-23187) in porcine basilar arteries. $\bar{x} \pm$ SD (ng / 100 mg tissue). $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$.

Dazoxiben(μmol / L)	n	Leukotrienes
0	9	1.7 ± 0.5
1	5	$1.2 \pm 0.3^{**}$
10	5	$0.9 \pm 0.4^{***}$
100	6	$0.6 \pm 0.4^{***}$

DISCUSSION

本文结果表明，呋喹氧苯对 0.5-50 μmol / L 对离体猪脑基底动脉产生 TXB_2 无影响，却能增加 PGI_2 的产生，相同浓度的吲哚美辛对基底动脉产生 TXA_2 亦无影响，但能显著抑制 PGI_2 的产生，而且不论有无外源性 AA，calcimycin 刺激，猪脑基底动脉所产生 TXB_2 无显著变化，这可能是由于 TXA_2 合成酶主要存在于血小板中，而在血管组织中量较少或活性较低有关[9,10].

本文结果还表明，呋喹氧苯对 1-100 μmol / L 可使基底动脉产生 LT 显著减少，对 AA 代谢的脂氧酶途经具有抑制作用. 结合前文[3] 结果，呋喹氧苯既能抑制 TXA_2 合成酶，使 TXA_2 减少，又能抑制 LT 的产生，对 AA 的两条代谢途经均有影响. 已经证明，脑缺血时脑内 TXA_2，LT 等物质可显著增加[11,14]. 本实验结果进一步阐明了呋喹氧苯治疗脑缺血的作用机理. 与吲哚美辛比较，后者虽能降低血浆中 TXA_2 水平，但同时亦降低 PGI_2 水平，又由于其抑制环氧酶，使脂氧酶代谢产物 LT 合成增加[4,15]，对保护脑缺血不利.

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蕊木宁对小鼠肝微粒体混合功能氧化酶的诱导作用

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Induction by kopsinine of hepatic mixed-function oxidase in mice

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ABSTRACT Kopsinine is an indole alkaloid. Oral administration of kopsinine 200 mg / kg once daily for 3 d significantly increased liver microsomal protein, cytochrome P-450, cytochrome b_5, NADPH-cytochrome C reductase, aminopyrine demethylase and benzo(a)pyrine hydroxylase activities in mice. kopsinine only induced cytochrome P-450 in rough endoplasmic reticulum of liver. SDS-polyamine gel electrophoresis analysis showed that the protein bands of microsomes from kopsinine treated mice were similar to that induced by phenobarbital in mice. Metyrapon, a specific inhibitor of cytochrome P-450, partially antagonized aminopyrine demethylase activity of microsomes from mice treated with kopsinine. The results suggest that kopsinine yields a pentobarbital-like induction on liver mixed function oxidase in mice. In addition, kopsinine was found to shorten the barbital-induced sleeping time in mice.

KEY WORDS kopsinine; microsomes; mixed function oxidases

提要 蕊木宁能拮抗 CCl_4 等毒物引起的小鼠肝损伤[1]. 本文报道蕊木宁 200 mg / kg, q d × 3, ig, 能显著增加小鼠肝微粒体蛋白质含量、细胞色素 P-450、NADPH-细胞色素 C 还原酶、细胞色素 b_5、氨基比林脱甲基酶及苯并芘羟化酶活性. 蕊木宁主要提高肝细胞粗面内质网细胞色素 P-450 含量. 可见, 蕊木宁对小鼠肝微粒体混合功能氧化酶有诱导作用.

关键词 蕊木宁; 微粒体; 混合功能氧化酶类

前文报告蕊木宁(kopsinine)对 CCl_4 引起的小鼠肝损伤及肝微粒体脂质过氧化有抑制作用, 细胞色素 P-450 特异性抑制剂甲吡酮(metyrapone)可部分地拮抗 CCl_4 的脂质过氧化作用[1,2], 提示肝微粒体细胞色素 P-450 与蕊木宁的抗肝损伤作用有一定关系. 鉴于具有抗肝损伤的联苯双酯和五味子素均有诱导肝微粒体细胞色素 P-450 的作用[3,4], 因此, 本文研究了蕊木宁对小鼠肝混合功能氧化酶的影响.

MATERIALS AND METHODS

昆明种小鼠, 100 只, ♂, 体重 20.2 ± SD 1.8 g. 还原型辅酶 II (nicotinamide adenine nucleotide phosphate, NADPH)细胞色素 C、葡萄糖-6-磷酸均购自 Sigma 公司.

小鼠肝微粒体的制备 小鼠禁食一夜, 次日断头处死, 取出肝脏, 制备肝微粒体[5]. -30℃保存.

肝细胞滑面和粗面内质网的分离 小鼠禁食, 次日处死, 将各组中每 3 只小鼠的肝脏合并, 称重, 制成肝匀浆, 分离滑面和粗面内质

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