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酮替芬对兔血小板聚集和大鼠中性粒细胞血小板激活因子生成的影响[1]

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Effects of ketotifen on rabbit platelet aggregation and platelet activating factor formation from rat neutrophils[1]

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ABSTRACT The effects of ketotifen (Ket) on rabbit platelet aggregation induced by platelet activating factor (PAF), ADP and arachidonic acid (AA) and PAF formation from A-23187-stimulated rat neutrophils *in vitro* were studied. PAF (15-100 pmol / L) induced rabbit platelet aggregations, with an EC_{50} of 33 pmol / L. Ket shifted the PAF dose-dependent platelet aggregation curve to the right in a parallel fashion with no depression of the maximal response and reversed the secondary aggregation phase, suggesting that Ket had competitive antagonistic activity against PAF-induced platelet aggregation. It also showed inhibitory effects on platelet aggregations induced by ADP 10 μmol / L and AA 50 μmol / L, the IC_{50} were 94.5 and 143.5 μmol / L respectively However, it failed to influence PAF formation from rat neutrophils stimulated by A-23187 2.5 μmol / L *in vitro*. The inhibitory effects of Ket on platelet activation, particularly PAF-induced platelet aggregation, may contribute to its anti-asthmatic properties.

KEY WORDS ketotifen; blood platelets; platelet activating factor; platelet aggregation; neutrophils

提要 PAF (15-100 pmol / L) 诱导兔血小板聚集呈明显剂量 - 效应关系, EC_{50} 为 33 pmol / L; Ket 竞争性拮抗 PAF 诱导血小板聚集, 较高浓度时还能抑制 ADP 及花生四烯酸诱导的血小板聚集. 但本文未能证实 Ket 对大鼠中性粒细胞 PAF 生成有抑制作用. Ket 的抗血小板聚集作用可能为其平喘作用机理之一.

关键词 酮替芬; 血小板; 血小板激活因子; 血小板聚集; 嗜中性白细胞

酮替芬 (ketotifen, Ket) 临床上用于预防哮喘急性发作, 但其预防机理至今尚未完全阐明. Ket 能有效地阻止血小板激活因子 (platelet activating factor, PAF) 诱导支气管收缩和气道反应性增高[1], 提示其平喘作用可能与抑制 PAF 有关. 为进一步阐明其平喘机理, 本文观察了 Ket 的抗兔血小板聚集作用以及对 A-23187 诱导大鼠中性粒细胞生成 PAF 的影响.

MATERIALS AND METHODS

Ket 由上海第十六制药厂生产. PAF,

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ADP、磷脂酶 A_2 (phospholipase A_2、PLA_2)、溶血卵磷脂(lysolecithin, LL)及神经鞘髓磷脂(sphingomyelin, S)均系美国 Sigma 公司产品. 花生四烯酸(arachidonic acid, AA)由瑞士 Fluka 公司生产. 卡西霉素(calcimycin, A-23187)为西德 Calbiochem-Behring 公司产品. 牛血清白蛋白(BSA)为中国科学院上海生物化学研究所产品. 角叉莱胶由辽宁省药物研究所制备.

兔洗涤血小板(WRP)制备 新西兰兔耳中央动脉取血, ACD(每 100 ml 含枸橼酸钠 2.5 g, 枸橼酸 1.5 g 以及葡萄糖 2.0 g) 抗凝(6 : 1, vol / vol), 500 × g 离心 15 min 制得富含血小板血浆(PRP). PRP 经 4℃无钙明胶台氏液洗涤两次后混悬于 37℃明胶台氏液, 调节血小板数至 2.5×10^8 / ml.

无钙明胶台氏液组成(mmol / L): KCl 2.6, NaCl 136.7, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, glucose 5.0, EGTA 0.2, 每 1000 ml 加明胶 2.5 g, pH 6.5.

血小板聚集试验 Ket 5-10 μl 于聚集诱导剂前 1 min 加入 200 μl WRP (37℃)中, 聚集诱导剂加入体积为 1-10 μl, 按比浊法[2]在血小板聚集仪(PAM-2 型, 江苏丹阳无线电厂)上测定血小板聚集反应. 以明胶台氏液的透光度为 100, 聚集率(AR)%即为加入聚集剂后所能达到的最大透光度的百分率. 聚集抑制率 = (对照管 AR - 实验管 AR) / 对照管 AR.

中性粒细胞悬液中 PAF 的提取和测定 中性粒细胞取自角叉莱胶致炎的 Sprague-Dawley 大鼠胸腔渗出液[3], 混悬于 BSA - 台氏液中(含 BSA 2.5 g / L)并调节至一定细胞数. 1 ml 细胞悬液 37℃预孵 10 min, 加 A-23187 2.5 μmol / L 攻击 15 min, 置冰浴终止反应, 按 Bligh-Dyer 法[4]加以改良提取 PAF: 加 0℃甲醇 2.5 ml (含 2 %冰乙酸)和 0℃氯仿 1.25 ml 于细胞悬液中, 充分振摇后置冰浴放置 0.5 h(每 5 min 振摇一次); 离心(1500 × g, 15 min)弃去沉淀物, 加氯仿和蒸馏水各 1.25 ml 于上清液中, 充分振摇后静置 0.5 h, 等待分层; 吸取氯仿层液体负压抽干, 加 BSA-台氏液 100 μl 溶解之, 此即 PAF 提取液, -20℃保存. 用血小板聚集法生物测定提取液中 PAF 的含量.

观察 Ket 对 PAF 生成的影响时, Ket 先与细胞悬液 37℃作用 10 min, 洗涤两次去除胞外药物后加 A-23187 2.5 μmol / L 攻击并提取 PAF.

上述 PAF 提取液经薄层色谱(TLC)证实与 PAF 标准品有相同 R_F 值, 其血小板聚集活性能被 PLA_2 完全灭活, 与文献[5, 6]报道一致, 被鉴定为 PAF.

PAF 标准液配制: PAF 溶于氯仿 - 甲醇溶液(9 : 1, vol / vol), -20℃保存. 使用时取 10 μl 负压抽干, 再溶于一定量 BSA - 台氏液至需要浓度, 24 h 内使用.

RESULTS

对 PAF 诱导兔血小板聚集的竞争抑制 PAF(15-100 pmol / L)诱导兔洗涤血小板聚集呈明显剂量 - 效应关系, EC_{50} 为 33 pmol / L. Ket 5 μmol / L 可使 PAF 诱导血

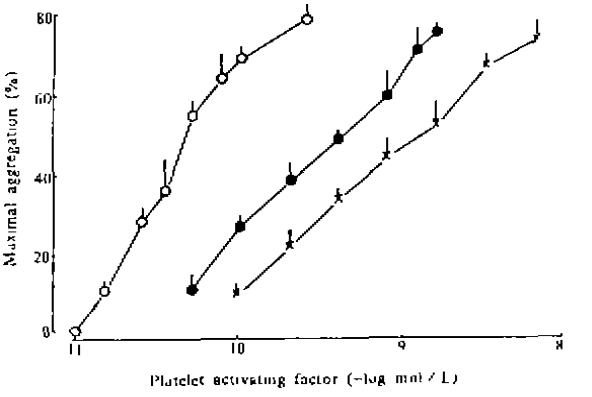

Fig 1. Concentration-dependent platelet aggregation induced by platelet activating factor in the presence of ketotifen 0 (○, n = 4), 5 (●, n = 3) and 20 (×, n = 3) μmol / L. $\bar{x} \pm SD$.

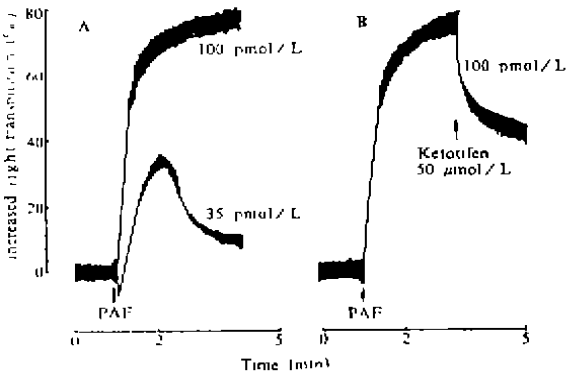

Fig 2. PAF-induced rabbit platelet aggregations without (A) or with (B) ketotifen.

小板聚集的量－效曲线平行右移，而最大反应不降低(Fig 1)；Ket 20 μmol／L 使 PAF 的量－效曲线进一步右移(Fig 1). Ket 50 μmol／L 则使 PAF 100 pmol／L 诱导的血小板聚集第二相完全逆转(Fig 2). 上述结果表明 Ket 能竞争性拮抗 PAF 诱导血小板聚集.

对多种诱导剂的血小板聚集作用的影响 PAF，ADP 和在花生四烯酸(AA)诱导血小板聚集，最大聚集率达 85 ± 5% ($\bar{x}$ ± SD) 的浓度分别为 0.5 nmol／L，10 μmol／L 和 50 μmol／L. Ket 抑制 PAF 0.5 nmol／L 诱导的血小板聚集，其 IC_{50} 为 3.2 μmol／L；浓度较高时还抑制 ADP 10 μmol／L 和 AA 50 μmol／L 诱导的血小板聚集，IC_{50} 分别为 94.5 和 143.5 μmol／L.

对 A-23187 诱导大鼠中性粒细胞生成 PAF 的影响 在没有 Ket 存在时，A-23187 2.5 μmol／L 可刺激大鼠中性粒细胞生成 PAF，PAF 生成量随细胞数增加而显著增高，5×10^6、1×10^7、2×10^7 中性粒细胞分别产生 81 ± 24(n= 6)、282 ± 82 (n= 9)和 883 ± 246 (n= 6) pg PAF ($\bar{x}$ ± SD). 中性粒细胞 2×10^7 分别与 Ket 20、100 或 500 μmol／L (最终浓度)作用 10 min 后再加 A-23187 2.5μmol／L 攻击，PAF 生成量与对照组无显著差异(Tab 1).

实验中发现，中性粒细胞 2×10^7 经与 Ket 1.0 mmol／L 或更高浓度 37℃ 作用 10 min 并洗涤两次后，台盼蓝染色显示细胞存活率低于 10 %，而 Ket 500 μmol／L 或更低浓度则不影响中性粒细胞活性.

Tab 1. Effect of ketotifen on A-23187 (2.5 μmol／L)-induced PAF formation from rat neutrophils (NP). $\bar{x}$ ± SD. * $P > 0.05$ *vs* control.

ketotifen (μmol／L)	n	PAF(pg／2×10^7 NP)
0	4	597 ± 414
20	3	858 ± 844*
100	4	702 ± 447*
500	4	781 ± 425*

DISCUSSION

本文工作以兔洗涤血小板为标本，发现 Ket 使 PAF 诱导的血小板聚集量－效曲线平行右移而不降低最大反应，并使血小板第二相聚集逆转，表明 Ket 能竞争性拮抗 PAF 活化血小板；提高 Ket 浓度则对 ADP 及 AA 诱导的血小板聚集也有抑制作用. 血小板活化是哮喘时支气管收缩和气道高反应性产生的基础[7,8]，推测 Ket 的抗血小板活化作用，尤其是拮抗 PAF 诱导血小板聚集，至少能部分解释其平喘机制.

本文还研究了 Ket 对来自大鼠胸腔的中性粒细胞在 A-23187 刺激下 PAF 生成的影响，发现 Ket 不抑制 PAF 的生成，即使 Ket 浓度高达 500 μmol／L，与 Joly 等[9]报道不一致，他们发现 Ket 能抑制由单克隆 IgE 被动致敏的小鼠骨髓肥大细胞受相应抗原攻击时 PAF 的生成，IC_{50} 为 20 μmol／L. 本文结果与 Joly 等[9]报道的差异可能与所用刺激剂不同有关，因为 Ket 抑制抗原诱导的小鼠肥大细胞 Ca^{2+} 内流，而不影响 A-23187 诱导的 Ca^{2+} 内流[9]. 哮喘发作时体内可产生较多 PAF[10,11]，Ket 是否能影响哮喘时体内 PAF 的产生尚有待研究.

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3,4,5-三羟基芪-3-β-单-*D*-葡萄糖苷在体外对兔血小板聚集和产生血栓素 B_2 的影响[1]

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Influences of 3,4,5-trihydroxystibene-3-β-mono-*D*-glucoside on rabbits' platelet aggregation and thromboxane B_2 production *in vitro*

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ABSTRACT Platelet aggregation and thromboxane B_2 (TXB_2) production induced by arachidonic acid (AA) or Adenosine diphosphate (ADP) were studied by turbidimetry and radioimmunoassay in rabbits. 3,4,5-tri-hydroxystibene-3-β-mono-*D*-glucoside (PD) 6.7-107.2 μmol/L inhibited platelet aggregation and the production of TXB_2 as well. The inhibitions by PD were dose-dependent : 48-90 % (AA-induced) and 43-69 % (ADP-induced) for platelet aggregation; 50-87 % (AA-induced) and 43-68 % (ADP-induced) for TXB_2. There were positive correlations between the inhibition of platelet aggregation and production of TXB_2.

KEY WORDS glucosides; adenosine diphosphate; arachidonic acids; thromboxane B_2; platelet aggregation; nephelometry and turbidimetry; radioimmunoassay

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