Biochim Biophys Acta 1980; 604 : 191

14 Moskowitz N, Andrés A, Silva W, Shapiro L, Schook W, Puszkin S. Calcium-dependent binding of calmodulin to phospholipase A_2 subunits induces enzymatic activation. *Arch Biochem Biophys* 1985; 241: 413

15 Li J, Jiang YY, Yue TL. Inhibition of release of prostaglandins and leukotrienes from calcimycin-induced mouse peritoneal macrophages and bovine aorta endothelial cells by anisodamine. *Acta Pharmacol Sin* 1989; 10 : 274

中国药理学报 *Acta Pharmacologica Sinica* 1990 May; 11 (3) 285-288:

中性粒细胞对肥大细胞组胺释放的影响[1]

王瑶生、卞如濂、周汉良 (浙江医科大学药理教研室, 杭州 310006, 中国)

Effects of neutrophils on histamine release from mast cells

WANG Yao-Chen, BIAN Ru-Lian, ZHOU Han-Liang (*Department of Pharmacology, Zhejiang Medical University, Hangzhou 310006, China*)

ABSTRACT To determine whether neutrophils (NP) contribute to immediate anaphylaxis, the effects of NP on histamine release from mast cells (MC) were studied and changes in the NP morphology were investigated. When rat peritoneal MC and pleural NP collected from actively sensitized rats were mixed and challenged with antigen (trichosanthin), histamine release from MC was significantly increased. This promotive effect of NP on antigen-induced histamine release from MC was dosage-dependent. The observation of scanning and transmission electron microscopy showed that NP were activated and secretion was resulted. It is suggested that NP may be activated in immediate anaphilaxis and secrete some mediators which could promote histamine release from MC. Quercetin may inhibit the promotive effect of the NP on histamine release and the secretion of the NP while ketotifen and isoproterenol have no influence on this promotive effect.

KEY WORDS neutrophils; mast cells; histamine; quercetin; trichosanthin; electron microscopy

提要 中性粒细胞 (NP) 可以促进抗原诱导的致敏大鼠的肥大细胞(MC)组胺释放. 其促进作用与 NP 的数量呈依赖关系. 同时, NP 形态上表现出活性增强及分泌反应. 提示 NP 可以通过释放某些介质促进 MC 组胺释放. 槲皮素可以抑制 NP 对 MC 组胺释放的促进作用以及 NP 形态的变化.

关键词 中性粒细胞; 肥大细胞; 组胺; 槲皮素; 天花粉蛋白; 电子显微镜检查

中性粒细胞 (neutrophil, NP) 与迟发型哮喘反应及气道高反应性有密切关系[1]. 但是, 对 NP 在速发型变态反应中的作用了解甚少. 本文观察 NP 对肥大细胞 (mast cell, MC) 组胺释放的影响及槲皮素 (quercetin) 等药物对于 NP 有关功能的影响. 这将有助于了解 NP 在速发型变态反应中的作用. 进一步阐明过敏性哮喘的发病机理. 为寻找防治哮喘的有效药物提供依据.

MATERIALS AND METHODS

Sprague-Dawley 大鼠, ♀♂兼用, 体重 201 ± SD 13 g (本校动物科). 角叉菜胶 (carrageenin, 辽宁药物研究所), 天花粉蛋白 (trichosanthin, 上海有机化学研究所), 槲皮素 (上海试剂二厂), 酮替芬(ketotifen, 上海第十六制药厂), 盐酸异丙肾上腺素(isoproterenol, 上海信谊制药厂).

大鼠致敏 Sprague-Dawley 大鼠, 每批 10 只, 共 8 批. 每只脚掌注射 5 mg/ml 天花粉蛋白悬液 0.1ml(悬混于氢氧化铝 4%凝胶

Received 1988 Dec 29 Accepted 1990 Jan 3
[1]Project supported by the National Natural Science Foundation of China, № 3861201

中), 后腿 im 2 × 10^{10} 灭活百日咳杆菌为佐剂. 3 wk 后, 收集胸腔 NP 和腹腔 MC.

细胞收集[2,3] 乙醚麻醉大鼠, 在大鼠右侧胸腔内注入 0.2% 角叉菜胶 0.2 ml. 4 h 后, 颈动脉放血处死大鼠, 用 37 ℃ Dulbecco's 磷酸缓冲液 (PBSS) 冲洗两侧胸腔, 收集胸腔冲洗液, 低渗破碎除去红细胞. 涂片检查 (Wright 染色): NP 纯度高于 95%, 台盼蓝染色活细胞占 95 %以上.

大鼠处死后, 37 ℃ PBSS 冲洗腹腔, 收集腹腔冲洗液, 用 56% (wt / vol) 牛血清白蛋白作为密度梯度分离剂, 将 MC 分离, 纯化. 涂片检查 (中性红染色): MC 纯度高于 95%, 脱颗粒细胞少于 5%.

MC 的组胺释放及测定[4,5] 试管内加入主动致敏的 MC 1 × 10^5 / ml, 不同数量的 NP, 磷酯酰丝氨酸 10 μg / ml, 最终容量 1 ml. 37℃ 孵育 10 min. 置冰浴, 加入 2 ml 冰 PBSS 终止反应. 组胺测定采用荧光分光光度法(日立 MPF-4 型荧光分光光度计).

药物对于 NP 促进 MC 组胺释放的影响 试管内加入适量主动致敏的 NP, 分别加入各种浓度的槲皮素(DMSO 溶解, 占反应液体积 1%), 酮替芬, 异丙肾上腺素, 37℃ 孵育 30 min. 置冰浴, 用冰 PBSS 将细胞洗两遍; 取经药物处理过的 NP 4 × 10^6 / ml 与 MC 1 × 10^5 / ml 混匀后作组胺测定.

电镜观察 试管内加入适量主动致敏的 MC, NP(或槲皮素处理过的 NP),磷酯酰丝氨酸及抗原, 37℃ 孵育 5 min, 按透射电镜, 扫描电镜标本常规处理后, 作透射电镜 (Philips EM 410 型), 扫描电镜 (日立 S-450 型) 观察, 摄片.

RESULTS

NP 对于抗原诱导的 MC 组胺释放的影响 致敏大鼠腹腔 MC 受到 20 μg / ml 天花粉蛋白抗原攻击后释放组胺. 在这个反应中加入不同数量致敏大鼠胸腔 NP, MC 组胺释放量明显增加. 增加效应与 NP 数量呈依赖关系(Tab 1). 改变抗原攻击剂量, NP 促进 MC 组胺释放作用仍可表现(Tab 2). 说明 NP 对抗原诱导的 MC 组胺释放有促进作用.

Tab 1. Effect of neutrophils (NP) on antigen-induced histamine release from mast cells (1 × 10^5 / ml). Antigen (trichosanthin) = 20 μg / ml. $n=6$, $\bar{x}$ ± SD. $^{***}P<0.01$ vs control.

10^{-5} × NP per ml	Histamine release (%)	Promotive rate (%)
0	25 ± 4	
5	31 ± 3***	22.9
10	38 ± 8***	50.3
20	46 ± 10***	84.7
50	53 ± 11***	111.3
100	56 ± 5***	122.2

Tab 2. Effect of NP (2 × 10^6 / ml) on antigen-induced histamine release from mast cells (MC, 1 × 10^5 / ml). $n=5$, $\bar{x}$ ± SD. $^{***}P<0.01$.

Group	Trichosanthin (μg / ml)	Histamine release(%)	Promotive rate(%)
MC	10	6.4 ± 3.3	
MC+NP	10	12.8 ± 2.3***	99.7
MC	20	16.6 ± 5.7	
MC+NP	20	34.0 ± 4.8***	105.7

药物对于 NP 促进 MC 组胺释放作用的影响 致敏大鼠 NP 与槲皮素、酮替芬、异丙肾上腺素共同孵育并清洗后加入致敏大鼠 MC, 用 20 μg / ml 抗原攻击. 随着孵育 NP 的槲皮素剂量增大, MC 组胺释放减少, NP 促进 MC 组胺释放的作用减弱(Tab 3). 表明槲皮素可以抑制 NP 促进 MC 组胺释放的作用. 而酮替芬 235, 1175 μmol / L, 异丙肾上腺素 0.1, 0.5 μmol / L 对 NP 促进 MC 组胺释放的作用无明显抑制.

抗原诱导的 NP 形态学的变化

1 扫描电镜 致敏 NP 与 MC 混合后, 经抗原攻击, NP 表面皱折加深加宽, 形成许多突起小泡, 类似分泌颗粒(Fig 1 B). 槲皮素 50 μmol / L 可抑制 NP 受到抗原攻击后的分

Tab 3. Influence of quercetin on promotive effect of NP (4×10^6/ml) on histamine release from MC. $n=5$, $\bar{x} \pm SD$. $^{***}P<0.01$.

NP	Quercetin (μmol/L)	Histamine release(%)	Promotive rate(%)
0	0	17.0±1.5***	
NP	0	49.2±2.5	190
NP	10	28.5±1.0***	69
NP	50	20.2±1.7***	19
NP	250	8.0±1.6***	−53

泌反应，NP 表面皱折较正常 NP 略有加深，但无明显颗粒状突起和圆形小泡(Fig 1 C).

2 **透射电镜** 致敏 NP 与 MC 混合后经抗原攻击，NP 浆膜形成许多突起，伪足，局部膜溶解，胞浆内容物外排，胞浆内颗粒释放，胞浆出现空泡(Fig 1 E). 槲皮素 50 μmol/L 可以抑制抗原诱导的 NP 形态变化，使 NP 形态基本正常(Fig 1 F).

DISCUSSION

MC 是速发型过敏反应的靶细胞，MC 释放的组胺是引起过敏反应的主要介质. 本文首次采用致敏大鼠同体细胞进行 NP 对 MC 组胺释放影响的研究，结果表明：致敏大鼠的 NP 可促进抗原诱导的 MC 组胺释放；电镜观察也证实致敏 NP 受抗原攻击后形态改变，活性增强，出现分泌反应. 说明 NP 在抗原攻击下可以释放某些介质，促进 MC 组胺释放. 在抗原直接作用下 NP 促进 MC 组胺释放的事实提示 NP 不仅能扩大速发型变态反应的炎症反应，参与迟发性哮喘的发生[6]，还可能直接参与了速发型变态反应的发生. 根据文献报道：可溶性刺激主要诱导 NP 分泌特殊颗粒及新生型介质[7]，新生型介质中氧化代谢产物可促进 MC 组胺释放[4]，推测 NP 促进 MC 组胺释放作用可能与 NP 释放的新生型介质有关.本实验中主要测定指标为 MC 介质组胺，因而 NP 接触抗原都在 MC 存在的条件下，故也不能排除 MC 经抗原激活后释放介质激活 NP，NP 再释放介质对 MC 起正反馈作用的可能. 进一步用抗原单独攻击 NP 并分析反应液成分的实验可解决上述问题.

本文发现黄酮(flavone)类衍生物槲皮素可以抑制 NP 对抗原诱导的 MC 组胺释放的促进作用. 电镜观察槲皮素对抗原诱导的 NP 分泌反应有抑制作用. 槲皮素具有抗过敏作用，可以抑制 5-脂氧酶的活性[8]，脂氧酶的活性是维持 NP 正常功能所需要的. 槲皮素也能抑制 MC 过敏介质的释放[9]. 但本实验未能阐明槲皮素的作用机理. 常用平喘药酮替芬、异丙肾上腺素都能抑制抗原诱导的 MC 介质释放，本实验中较大剂量的酮替芬，异丙肾上腺素对 NP 促进 MC 组胺释放作用无明显抑制，表明它们不能抑制大鼠 NP 某些介质的释放，提示大鼠 NP 在对这两种药物膜稳定作用的反应性方面与 MC，淋巴细胞等其它敏感细胞有差异.

REFERENCES

1 Moqbel R, Durham SR, Shaw RJ, *et al.* Enhancement of leukocyte cytotoxicity after exercise-induced asthma. *Am Rev Respir Dis* 1986; **133**: 609

2 Vinegar R, Truax JF, Selph JL. Some quantitative temporal characteristics of carrageenin-induced pleurisy in the rat. *Proc Soc Exp Biol Med* 1973; **143**: 711

3 Pearce FL, Ennis M. Isolation and properties of mast cell from the mesentry of the rat and guinea pig. *Agents Actions* 1980; **10**: 124

4 Coble BI, Lindroth M, Molin L, Stendahl O. Histamine release from mast cells during phagocytosis and interaction with activated neutrophils. *Int Arch Allergy Appl Immunol* 1984; **75**: 32

5 Shore PA, Burkhalter A, Cohn VH Jr. A method for the fluorometric assay of histamine in tissues. *J Pharmacol Exp Ther* 1959; **127**: 182

6 Larsen GL, Wilson MC, Clark RAF, Behrens BL. The inflammatory reaction in the airways in an animal model of the late asthmatic response. *Fed Proc* 1987; **46**: 105

7 Baggiolini M, Dewald B. The neutrophil. *Int Arch Allergy Appl Immunol* 1985; **76 (Suppl 1)**: 13

8 Yoshimoto T, Furukawa M, Yamamoto S, Horie

T, Watanabe-kohno S. Flavonoids: potent inhibitors of arachidonate 5-lipooxygenase. *Biochem Biophys Res Commun* 1983; **116**: 612

9 Ennis M, Atkinson G, Pearce FL. Inhibition of histamine release induced by compound 48/80 and peptide 401 in the presence and absence of calcium. Implications for the mode of action of anti-allergic compounds. *Agents Actions* 1980; **10** : 222

中国药理学报 *Acta Pharmacologica Sinica* 1990 May; **11** (3) : 288

Cisplatin and 5-FU infusions in therapy of advanced squamous cell carcinoma of head and neck in 50 Indian patients

S C SHARMA, A K SHARMA, K C SINGHAL

(Departments of ENT and Pharmacology, J N Medical College, A M U, Aligarh 202002, India)

ABSTRACT Fifty patients of advanced squamous cell carcinoma of head and neck received infusions of cisplatin 50 mg and 5-FU 500 mg weekly for 4 wk. At the end of chemotherapy, complete remission was seen in 14, partial remission in 25, minor remission in 5, and no response in 6 patients.

KEY WORDS head and neck neoplasms; squamous cell carcinoma; cisplatin; fluorouracil; intravenous infusions

Squamous cell carcinoma of head and neck represents more than 6% of the malignancies in the body. High response rates were obtained with cisplatin and 5-fluorouracil (5-FU) as initial treatment.[1,2] This study attempted to verify their effectiveness in India.

PATIENTS AND MEDICATIONS

Fifty patients (41 M, 9 F, aged 36-73 yr, median 56 yr) of advanced squamous cell carcinoma of head and neck in the ENT ward were treated in 1986. The tumor was located in larynx in 21, hypopharynx in 8, sinuses in 7, oropharynx in 6, oral cavity in 5, and nasopharynx in 3 patients. According to the American Joint Committee for Cancer Staging (1978), 28 patients were of stage III and 22 of Stage IV. Histological examination revealed well differentiated in 8, moderately differentiated in 21, poorly differentiated in 14, and undifferentiated in 7 patients. No evidence of distant metastasis was detected. Patients underwent detailed laboratory investigations such as hemogram, platelet counts, blood urea, serum creatinine, and serum bilirubin.

Diuresis was initiated by intravenous infusion of 2 L of 5% dextrose over 10-12 h before medication. Cisplatin 50 mg in 2 L of saline containing 50 ml of mannitol were infused in the next 6-8 h, followed by 5-FU 500 mg in 20 ml of water Antiemetics were injected intramuscularly in case of vomiting. The infusion was repeated weekly for 4 wk. After completion of the chemotherapy the tumor sizes were evaluated.

RESULTS

Complete remission (complete disappearance of tumor) was seen in 14, partial remission (> 50% reduction of tumor) in 25, minor remission (< 50% reduction) in 5, and no response in 6 patients. The majority of complete remissions were those with clinically movable nodes. Out of the 25 patients with partial remission, 4 had a complete disappearance at the primary site but had persistent regional nodes. There seemed to have no relation between the cell differentiation and the response. The tumors of oropharynx showed the best result.

The number of patients who developed adverse reactions: vomiting 33, leukopenia 17, stomatitis 10, diarrhea 8, thrombocytopenia 3, hearing loss 2, and alopecia 1.

REFERENCES

1 Kish J, Drelichman A, Jacobs J, *et al*. Clinical trial of cisplatin and 5-FU infusion as initial treatment for advanced squamous cell carcinoma of the head and neck. *Cancer Treat Rep* 1982; **66** : 471

2 Lim F, Duncan L, Coates A. Treatment of advanced squamous cell carcinoma of head and neck with cisplatin and continuous 96 hour infusion of 5-fluorouracil. *Aust N Z J Med* 1989; **19** : 83

Received 1989 Nov 21 Accepted 1989 Feb 27