

## 2-萘-3-(3,4-二甲氧基)苯-丙烯酸对兔血小板花生四烯酸代谢的影响

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### Effect of 2-naphthyl-3-(3,4-dimethoxy) phenyl-propenoic acid on the metabolism of arachidonic acid in rabbit platelets

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**ABSTRACT** Washed rabbit platelets were incubated with [<sup>14</sup>C] arachidonic acid (AA sodium salt) after being exposed to 2-naphthyl-3-(3,4-dimethoxy) phenyl-propenoic acid (NMPA), indomethacin (Ind) and imidazole (Imi) or control solvent.

The metabolites of AA in rabbit platelets were separated with the system of chloroform: methanol: acetic acid: water (90:8:1:0.8) by thin layer chromatography and quantitated by liquid scintillation counter.

The metabolism of AA was influenced by NMPA dose-dependently in the range of 0.05-0.5 mmol/L. The formation of thromboxane B<sub>2</sub> (TXB<sub>2</sub>) and 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT), the final metabolites in the pathway of cyclooxygenase-thromboxane synthetase, were decreased from 24 ± 6.7 to 3.2 ± 1.6% and 10.3 ± 2.49 to 4.7 ± 2.8%, respectively. Meanwhile, 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE), the metabolite in the pathway of lipoxygenase, was increased from 11.9 ± 1.7 to 34 ± 5.6%.

It is suggested that NMPA blocks the pathway of cyclooxygenase-thromboxane synthetase and then changes the direction of arachidonate metabolism in platelets, the activation of platelets is inhibited in this way.

**KEY WORDS** 2-naphthyl-3-(3,4-dimethoxy) phenylpropenoic acid; blood platelets; arachidonic acids/metabolism; radiochemistry; thin layer

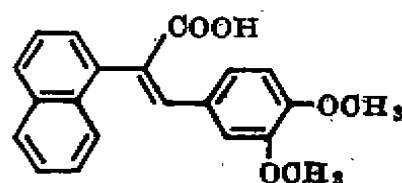
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chromatography; phenylpropenoic acids

**摘要** 体外放射化学方法研究表明, 2-萘-3-(3,4-二甲氧基)苯-丙烯酸(NMPA)在终浓度 0.05-0.5 mmol/L 范围内可使兔血小板环加氧酶-血栓素合成酶系终产物 TXB<sub>2</sub> 的生成率由 23.8% 降至 3.17%, 效应与剂量相关, 推测 NMPA 对该酶系有抑制作用, 从而使代谢途径改道, 通过此种作用而抑制血小板的活化。

**关键词** 2-萘-3-(3,4-二甲氧基)苯-丙烯酸(NMPA), 血小板; 花生四烯酸类/代谢; 放射化学; 薄层色谱法; 苯丙烯酸类

苯丙烯酸类化合物对花生四烯酸-前列腺素代谢有影响。丁香酚(eugenol)可抑制 PGE<sub>1</sub> 的生成<sup>(1)</sup>, 亦可降低血小板 TXB<sub>2</sub> 的生成率<sup>(2)</sup>, 阿魏酸(ferulic acid)可改变 TXA<sub>2</sub> 与 PGI<sub>2</sub> 的比值<sup>(3)</sup>。本实验室发现 2-萘-3-(3,4-二甲氧基)苯-丙烯酸(2-naphthyl-3-(3,4-dimethoxy) phenyl-propenoic acid, NMPA)亦可抑制不同诱导剂所致的血小板聚集, 并亦可改变 TXA<sub>2</sub> 与 PGI<sub>2</sub> 的比值<sup>(4)</sup>。现应用体外放射化学方法对该化合物对血小板花生四烯酸代谢的影响进行研究, 以期了解该类化合物抗血小板活化的可能的机理。



2-Naphthyl-3-(3,4-dimethoxy) phenyl-propenoic acid (NMPA)

### MATERIALS AND METHODS

[<sup>14</sup>C]花生四烯酸([<sup>14</sup>C]arachidonic acid, [<sup>14</sup>C]AA, Amersham 公司), 比活度为 2.21

GBq/mmol, TXB<sub>2</sub>, PGA<sub>2</sub>, PGD<sub>2</sub>, PGE<sub>2</sub>, PGF<sub>2</sub> 均为 F Needleman 教授赠送; 吲哚美辛 (Indomethacin, Ind, Sigma 公司); 咪唑 (imidazole, Imi) 为首都医学院申京建赠送; NMPA 由中国药科大学曹观坤教授合成提供。

LB-2772-2 型放射薄层扫描仪为 Berthold 公司产品; TRI-CABB 460-CD 型液体闪烁计数仪为 Packard 公司产品; 硅胶 G 板为浙江黄岩人民化工厂产品, 用前于 110℃ 活化 30 min. 接触血小板的玻璃器皿均以 1% 硅油 甲苯溶液处理。

新西兰兔, ♂, 5 只, 体重 2.79±SD 0.21 kg, 北京试验动物中心提供。

**洗涤血小板的制备** 兔用 3% 戊巴比妥钠 [30 mg/(ml.kg), iv] 麻醉, 自腹主动脉采血, 以 EDTA-Na<sub>2</sub> 77 mmol/L 抗凝, 抗凝剂与全血比例为 2.6:35. 将抗凝血以 200×g, 20℃ 离心 8 min, 吸取上层富血小板血浆 (platelet-rich plasma, PRP), 将 PRP 以 2200×g 离心 10 min, 沉降血小板, 除去血浆后以等容量的血小板洗液 (含 NaCl 0.15 mol/L: Tris-HCl, (pH 7.8), 0.15 mol/L: EDTA-Na<sub>2</sub> 77 mmol/L=90:8:2) 洗涤 2 次, 离心除去洗液, 将血小板重悬浮于无钙 Krebs 溶液中, 调节血小板数约为 2×10<sup>8</sup>/mm<sup>3</sup>.

**温育** 反应体系中含洗涤血小板 0.4 ml, 磷酸缓冲液 (PBS, pH 7.4) 0.4 ml, 药液或 PBS 0.2 ml. 将该体系于 37℃ 温育 20 min, 加入 0.05 ml [<sup>14</sup>C]AA (钠盐, 相当于 2×10<sup>5</sup> cpm), 继续温育 30 min, 然后加入甲酸 (2 mol/L) 0.1 ml 以终止反应。

**代谢物的提取、分离及定量**<sup>(5,6)</sup> 于上述温育反应体系中加入乙酸乙酯, 每次 1 ml 进行提取, 共 3 次; 合并有机相, 以氮气吹干. 将提取物溶于 25 μl 氯仿: 甲醇 (2:1) 混合液中, 取 20 μl 点样于硅胶 G 薄层层析板上, 同时点标准品. 用氯仿: 甲醇: 乙酸: 水 (90:8:1:0.8) 系统展开, 置碘缸中显色以确定标准品位置. 经放射薄层扫描及放射自显影定位后, 将

各相关斑点刮下, 置盛有 0.6 ml PPO 甲苯溶液的闪烁瓶内, 进行液闪计数, 计算各主要代谢产物的相对生成量 (生成率%), 相关代谢物斑点的计数 (cpm)/总放射计数 (cpm)×100%.

## RESULTS AND DISCUSSION

从 Fig 1 可见, 对照组代谢提取物有四个明显的斑点, R<sub>f</sub> 值分别为 0.61, 0.56, 0.53 和 0.22. 根据已有的标准品定位确认 R<sub>f</sub> 值为 0.61 和 0.22 的斑点为 AA 和 TXB<sub>2</sub>. 据文献所载<sup>(6)</sup>, 在相同展开条件下, 12-羟基花生四烯酸 (12-hydroxy-5,8,10,14-eicosatetraenoic acid, 12-HETE) 与 AA 的相对 R<sub>f</sub> 值 (R<sub>f</sub>' = R<sub>f</sub> HETE/R<sub>f</sub> AA) 为 0.93 (0.79/0.85); 12-HETE 与 TXB<sub>2</sub> 的 R<sub>f</sub>' 值为 2.47 (0.79/0.32). 而上述 0.56/0.61 和 0.56/0.22 分别为 0.92 和 2.54, 与此两个 R<sub>f</sub>' 值相近. 故可认为 R<sub>f</sub> 值为 0.56 的斑点代表 12-HETE. 以同法可确认 R<sub>f</sub> 值为 0.53 的斑点代表 12-羟基十七碳三烯酸 (12-hydroxy-5, 8, 10-heptadecatrienoic acid, HHT).

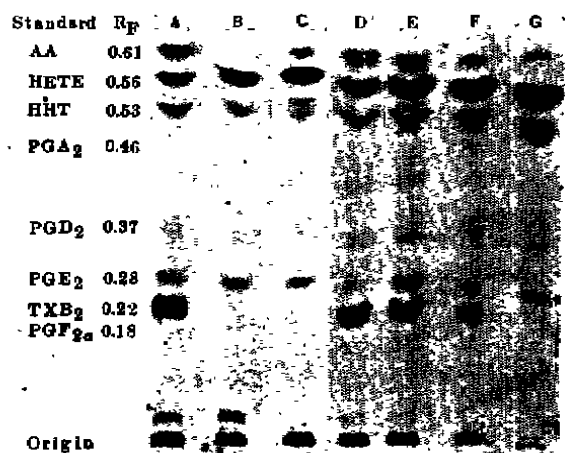


Fig 1. Autoradiogram of TLC separation of the metabolites after incubation of [<sup>14</sup>C]arachidonic acid with rabbit platelets in the presence of control solvent (column A), imidazole (column B), indomethacin (column C) and various concentration of NMPA (column D-G, 0.05, 0.1, 0.25 and 0.5 mmol/L, respectively).

由 Tab 1 可见, NMPA 在 0.05-0.5 mmol/

Tab 1. Effect of 2-naphthyl-3-(3,4-dimethoxy)phenyl-propenoic acid (NMPA), indomethacin (Ind) and imidazole (Imi) on the metabolism of [ $^{14}$ C]arachidonic acid in rabbit platelets, metabolites determined are thromboxane B<sub>2</sub> (TXB<sub>2</sub>), 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT) and 12-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE), n=4,  $\bar{x} \pm SD$ . \*P>0.05, \*\*P<0.05, \*\*\*P<0.01 vs control.

Drugs	Final concn (mmol/L)	Formation of metabolites (%)		
		TXB <sub>2</sub>	HHT	HETE
Control		24±6.7	10.3±2.5	11.9±1.7
Ind	0.03	1.7±0.6***	3.7±1.7**	31±9.5***
Imi	40	2.4±0.6***	8.0±1.7*	25±8.9**
NMPA	0.05	17±5.9*	10±5.1*	21±5.1**
	0.10	10.7±2.8**	8±6*	27±5.7***
	0.25	5.6±1.3***	6±4**	28±6.0***
	0.50	3.2±1.6***	4.7±2.8**	34±5.6***

L 范围内对兔血小板 [ $^{14}$ C]AA 的代谢有明显的影响。对环加氧酶-内过氧化物-血栓素合成酶途径的终产物 TXB<sub>2</sub> 及 HHT 的生成有抑制作用, 呈效应-剂量相关。伴随着上述途径代谢产物的减少, 脂加氧酶的代谢终产物 12-HETE 则显著增加, 亦呈剂量-效应相关。咪唑对 TXB<sub>2</sub> 的生成, 吲哚美辛对 TXB<sub>2</sub> 及 HHT 生成亦有十分显著的抑制作用。NMPA 的作用特点与咪唑不同而与吲哚美辛类似, 提示 NMPA 可能是环加氧酶抑制剂。

从实验结果推测 NMPA 的作用环节可能在于抑制 AA→内过氧化物→血栓素代谢转化。但究竟是抑制了环加氧酶还是特异性抑制 TXA<sub>2</sub> 合成酶尚待进一步研究。至于脂加氧酶的代谢产物 12-HETE 的显著增加, 则可能因为前述酶系被抑制时, 细胞内可被脂加氧酶利用的 AA 增加, 因此表现为代谢途径改道。本实验室亦曾发现<sup>(7)</sup>, 在相同浓度范围内 NMPA 并不能完全消除氧化代谢所产生的氧自由基, 表现为仅部分消除由 AA 所致的兔血小板化学发光, 这亦说明整个体系中的氧化代谢仍在进行, 只是代谢途径改变而已。另有一种观点认为<sup>(8)</sup>, 脂加氧酶的一个不稳定代谢物 HPETE 对该酶有正反馈作用, 而对血栓素合成酶则呈抑制作用。此观点在本实验中得到证实, 12-HETE 的生成增加, TXB<sub>2</sub> 生成抑制与 HHT 的生成抑制之间并不平行, 这样必然存在某种内

在的代谢反馈调节机制。此外, 由于血小板 AA 代谢的主要终产物并非经典的前列腺素<sup>(9)</sup>, 因此本实验尚无法确认 NMPA 究竟作用于哪一个酶, 有待于进行其它实验加以确定。

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## 5-氨基水杨酸甲酯盐酸盐对兔实验性溃疡性结肠炎的治疗作用

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### Therapeutic effect of methyl 5-aminosalicylate on experimental ulcerative colitis in rabbits

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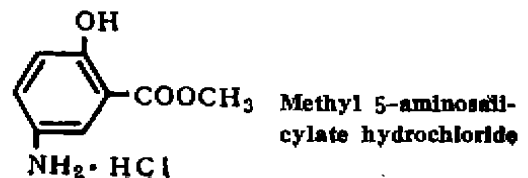
**ABSTRACT** Using the improved Kirsner model of ulcerative colitis in rabbits, we found that the therapeutic effect of methyl 5-amino salicylate hydrochloride (M-5-AS) was similar to that of sulfasalazine (salicylazosulfapyridine, SZ). The length of pathological damage and the range of diffuse ulcers were decreased in comparison with the control group ( $P < 0.01, 0.05$ ). Microscopically, tissue repairing at the regions of ulcers and proliferation in serolemma were noted, and the edema in muscular mesenchyme was less evident in the treated rabbits. M-5-AS completely inhibited the spontaneous contraction of rat stomach fundus strips and colon. The  $IC_{50}$  of the resting tone of the 2 tissues were  $1.30 \pm 0.39$  and  $0.72 \pm 0.25$  mmol/L, respectively. In addition, M-5-AS antagonized the contraction induced by human fresh semen. The results suggest that the therapeutic mechanism is related to the antagonism of prostaglandins in the inflammatory regions.

**KEY WORDS** salicylazosulfapyridine, amino-salicylic acids; ulcerative colitis; gastric fundus; colon

**摘要** 在兔溃疡性结肠炎模型上, 5-氨基水杨酸甲酯盐酸盐(M-5-AS)的疗效与柳氮磺吡啶(SZ)相似, 病变长度及范围明显缩小, 溃疡面修复, 炎症轻, 肌层间质轻度水肿, 浆膜层增生。M-5-AS抑制大鼠胃底肌条及结肠肌条的自发收缩, 其 $IC_{50}$ 分别为 $1.30 \pm 0.39$ 及 $0.72 \pm 0.25$  mmol/L, 并拮抗人新鲜精液的收缩作用, 提示本品疗效可能与拮抗PG有关。

**关键词** 柳氮磺吡啶; 氨基水杨酸; 溃疡性结肠炎; 胃底; 结肠

在柳氮磺吡啶(sulfasalazine, salicylazosulfapyridine, SZ)作为抗菌药应用过程中, 发现其对兔溃疡性结肠炎有治疗效应, 口服SZ分解为5-氨基水杨酸(5-amino-salicylic acid, 5-ASA)和磺胺吡啶, 后者常产生胃肠不适, 过敏反应, 包括发热, 皮疹和粒细胞减少症, 及可逆性男性不育<sup>(1)</sup>。为此有必要研制5-ASA的衍生物以取代SZ。近年来, 昆明制药厂合成了新化合物5-氨基水杨酸甲酯盐酸盐(methyl 5-aminosalicylate hydrochloride, M-5-AS)。本文报道用兔实验性溃疡性结肠炎模型研究M-5-AS的疗效, 并观察对大鼠离体胃底及结肠标本收缩反应的影响。



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