

# 大蒜素对实验性高胆固醇血症兔的抗血小板聚集作用

山东省医学科学院 张胜乐 徐英杰 陆 通 李 苙 高春义

**内容提要** 用兔制成实验性高胆固醇血症动物模型, 观察大蒜素对血小板聚集率、血小板内 cAMP 和 cGMP 等指标的动态变化。结果表明: 大蒜素具有明显抑制高胆固醇血症引起的血小板聚集作用, 这种作用是通过提高血小板内 cAMP 这一重要环节而实现的, 与 cGMP 无关。

1978 年 Bordia 等<sup>(1)</sup>首次发现大蒜精油有抑制血小板聚集作用。然而, 大蒜有效成分在高脂血症情况下抗血小板聚集效果以及作用机理尚不清楚。根据提高血小板内外环磷酸腺苷(cAMP)水平可抑制血小板聚集和释放反应<sup>(2)</sup>, 我们在高胆固醇血症动物模型上, 动态观察了合成大蒜素<sup>(3)</sup>(二烯丙基三硫化物)对血小板聚集性和血小板内环核苷酸(cAMP 和 cGMP)的影响, 验证大蒜有效成分抗血小板聚集作用并探讨其作用机理, 为人工合成大蒜素的临床应用提供依据。

## 材料和方法

### 一、动物分组和给药

新西兰兔(中国医学科学院动物中心)32只, 体重1.5~2.5 kg, 雌雄各半。按同窝配对方法分成三组: 对照组10只; 胆固醇组11只; 胆固醇加大蒜素组11只(简称大蒜素组)。颗粒饲料(烟台福山饲料厂)加少量蔬菜喂养。

胆固醇(Merck)每日0.5g/kg, 大蒜素(上海第二制药厂, 胶丸)根据预实验有效剂量, 每日20mg/kg。用注射器从胶丸中抽出, 按1%比例加兔胆汁稀释液(5%)乳化后, 经口灌入(其它组用胆汁稀释液做对照)。每周六次, 实验第11周起, 为观察药物在血胆固醇较高情况下的效果, 胆固醇和大蒜素组均加喂蛋黄粉(10%), 猪油(2%), 实验至15周止。定期测定各项指标。

### 二、血小板聚集率测定

基本采用 Velaskar<sup>(4)</sup>方法。用硅化试管及针头。空腹12小时, 取耳中央动脉血0.3ml 加入含有3.8%枸橼酸钠40μl 试管中。2分钟后, 移出0.2ml 于另一试管, 5分钟时, 加入肾上腺素(1mg/ml, 山东新

华制药厂)20μl。剧烈振动10秒, 30秒时, 取出3μl 涂薄血片。干燥后, W-G染色。于油镜下, 沿玻片长轴记录聚集和未聚集血小板数(总数不得少于200个), 用以下公式求出聚集率:

$$\frac{\text{聚集血小板数}}{\text{聚集血小板数} + \text{未聚集血小板数}} \times 100\%$$

### 三、血小板环核苷酸的提取和测定

参考张夏英等的方法<sup>(5,6)</sup>加以改进。试管、针头均硅化。空腹12小时, 取耳中央动脉血4ml 加80μl EDTA-Na(0.5M), 室温下离心(160g)7分钟, 取上清(富含血小板血浆, PRP)计量并计数。PRP离心(2000g)13分钟, 上清(贫血小板血浆, PPP)计数。沉淀加双蒸水0.5ml 混匀, -40°C快速冻融两次。加10%三氯醋酸0.25ml, 冰浴中15分钟, 离心(2000g)10分钟。上清用4倍水饱和乙醚提取5次后, 定量移入小烧杯中, 60°C水浴中吹干, 低温保存。

PRP 与 PPP 计数(万/μl)之差, 乘以 PRP μl 数, 再乘  $\frac{\text{提取液} \mu\text{l 数}}{0.75}$  求出小烧杯中相应血小板数。根据样品测定时稀释倍数, 算出每10<sup>9</sup>个血小板内环核苷酸的含量(pmol/10<sup>9</sup>)。

样品测定用本室制备的放射免疫测定盒, 按照文献<sup>(6)</sup>方法。Beckman 9800 型液闪仪计数。

### 四、血清胆固醇测定用硫酸—钼钼法。

## 结 果

动物一般情况: 实验1月后, 大蒜素组兔平均体重比胆固醇组稍高(150g左右)。持续到实验结束。第11周加喂蛋黄粉和猪油后, 第15周有4只兔(胆固醇组2只, 大蒜组2只)因出现黄疸被删除。

实验结果见附表。血清胆固醇第7周大蒜素组低于胆固醇组(P<0.001)。而11周加喂蛋黄粉后, 第14

附表 大蒜素对兔胆固醇血症时血小板聚集性及 cAMP 的影响 (M±SE)

	血清胆固醇(mg%)			血小板聚集率(%)			血小板 cAMP (pmol/10 <sup>9</sup> )		
	2周	7周	14周	1周	6周	13周	3周	8周	15周
I	82.4 ±2.90	84.70 ±3.93	99.44 ±10.71	20.60 ±3.33	23.30 ±1.97	32.40 ±1.79	7.19 ±0.45	8.10 ±0.59	7.05 ±0.91
(n)	(10)	(10)	(9)	(10)	(9)	(10)	(10)	(10)	(8)
II	88.09 ±3.65	112.60** ±6.19	795.50** ±31.71	22.30 ±2.27	31.50 ±3.31	38.45* ±2.07	6.90 ±0.56	5.43** ±0.42	5.99 ±0.91
(n)	(11)	(11)	(10)	(11)	(10)	(11)	(11)	(11)	(8)
III	87.54 ±3.79	80.45△△ ±3.93	745.45** ±49.98	24.00 ±1.89	22.45△ ±1.78	29.20△△ ±2.33	9.05 ±0.89	9.72△△ ±0.76	9.28△ ±1.13
(n)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(8)

注: \*, \*\*, 与 I 相比  $P < 0.05$  和  $< 0.01$ ; △, △△, 与 II 相比  $P < 0.05$  和  $< 0.01$ ; I: 对照组, II: 胆固醇组, III: 胆固醇加大蒜素组

周两组间无明显差别。血小板聚集率第 6 周大蒜素组与对照组接近, 低于胆固醇组 ( $P < 0.05$ ), 第 13 周, 对照组和大蒜素组都显著低于胆固醇组 ( $P < 0.05$ ,  $P < 0.01$ )。血小板 cAMP 第 8 周对照组与大蒜素组明显高于胆固醇组 ( $P < 0.01$ ,  $P < 0.001$ ), 第 15 周大蒜素组仍高于胆固醇组 ( $P < 0.05$ )。

血小板 cGMP 第 15 周大蒜素组为  $1.43 \pm 0.21$ ; 胆固醇组为  $1.03 \pm 0.21$ , 两组间无显著差别。对照组  $2.8 \pm 0.64$ 。

## 讨 论

多种动脉粥样硬化危险因素(包括物理、化学、免疫方面)均能引起血小板聚集。高胆固醇血症是其化学因素之一。已有动物实验表明<sup>①</sup>, 随着血脂升高, 心脏及血管中  $PGI_2$  (前列环素) 水平显著下降, 3 个月后又有一定程度回升。而血小板  $TXA_2$  (血栓素  $A_2$ ) 含量明显升高。说明高脂血症引起血小板聚集性升高是由于  $PGI_2/TXA_2$  比值下降, 进一步引起血小板内 cAMP 水平下降。

本实验表明, 血胆固醇水平升高伴有血小板聚集率升高和血小板内 cAMP 下降, 大蒜素具有提高血小板内 cAMP 作用并抑制血小板聚集。不少离体实验已证实<sup>②</sup>, 利用外源性或内源性方法提高血小板 cAMP 均有抑制血小板聚集作用。本实验结果经相关分析也表明血小板 cAMP 与聚集率之间呈负相关 ( $r = -0.42$ ,  $P < 0.05$ )。说明大蒜素抑制血小板聚集作用可能是由于提高了血小板内 cAMP 水平。cAMP 升高的原因有待进一步探讨。

一般血胆固醇升高伴随血小板聚集率升高和血小板 cAMP 降低。至于第 15 周胆固醇组血小板 cAMP 比对照组降低不够显著, 可能与实验后期动脉壁  $PGI_2$

合成回升<sup>③</sup>有关。第 14 周, 大蒜素组胆固醇比胆固醇组低的不够显著, 可能与 11 周加喂蛋黄粉后, 药物剂量相对不足有关。

血小板 cGMP 在大蒜素组与胆固醇组之间没有明显差别, 这与中药蒲黄的实验结果<sup>④</sup>近似。目前认为 cGMP 与血小板聚集和释放反应关系不大<sup>⑤</sup>。

(本工作受到本院许海修、李志荣、崔树岭同志的热情支持和帮助, 特此致谢)

## 参 考 文 献

1. Bordia A, et al. Effect of garlic on human aggregation in vitro. *Atherosclerosis* 1978; 30:355.
2. Salzman EW. Cyclic AMP and platelet function. *N Engl J Med* 1972; 286(1):258.
3. 郎彝江, 等. 大蒜有效成分的研究. *中草药* 1981; 1:42.
4. Velaskar DS, et al. A new aspect of platelet aggregation and a test to measure it. *Amer J Clin Pathol* 1982; 77:267.
5. 张夏英, 等. 一种灵敏的血小板内环磷酸腺苷测定法. *中华心血管杂志* 1980; 8(2):142.
6. 徐英杰, 等. 环磷酸腺苷(cAMP)放射免疫分析法的建立. *中国医学科学院年报* 1980:35.
7. Gryglewski RJ, et al. Prostacyclin and thromboxane  $A_2$  biosynthesis capacities of heart arteries and platelet at various stages of experimental atherosclerosis in rabbits. *Atherosclerosis* 1978; 31:285.
8. 徐也鲁, 等. 阿斯匹林、蒲黄对家兔动脉粥样硬化的防治及机理探讨. 全国首届动脉粥样硬化学术会议交流资料 (1984, 吉林).
9. Weiss A, et al. Platelet release reaction and intracellular cGMP. *Blood* 1978; 52(3):524.

### Studies on Pharmacological Experiment of *Corydalis Ambigua*

You Chunlai (尤春来), Wang Yiming (王义明)

Dept. of Pharmacology, Liaoning College of TCM, Shenyang

Using benzene extracted alkaloids of *Corydalis ambigua*, the effect of analgesia and its action on cardiovascular system were studied. This alkaloid was compared with that of *Corydalis yanhusuo* extracted in the same way. The following results were obtained:

1. The effect of analgesia: (1) Heating plate method: Mice were injected intraperitoneally with this extract 30 mg/kg and 45 mg/kg. 20 min. later, the responding time of algesia was markedly prolonged and sustained for 90 min. In comparison with the same dose of *C. yanhusuo*, it has the similar effect. (2) Method of cramping in mice: Mice were injected subcutaneously with the same dose of the extract. The frequency of cramping was  $7.5 \pm 6.3$  and 0 within 15 min. respectively. As compared with same dose of *C. yanhusuo*, the frequency of cramping was  $15.5 \pm 9.5$  and  $12.2 \pm 7.0$ . Therefore this extract was more potent than that of *C. yanhusuo*.

2. Experiment on anoxia-tolerance in mice: The experimental group was administered intraperitoneally with the same dose of the extract, and showed markedly extended survival time for 72% and 142% respectively. The group of *C. yanhusuo* with the same dose was 8.8% and 28% only.

3. The effect on cardiovascular system: (1) Perfused experiment of isolated rabbit's heart: Either this extract or *C. yanhusuo* administered same dose (0.3 mg and 1.0 mg) would inhibit the myocardial contraction significantly, and increase the coronary flow markedly. (2) Experiment of acute myocardial ischemia: The elevating ST-T wave of lead  $V_3$  produced by pituitrin-induced ischemia in guinea pigs was opposed by intravenous injection 15 mg/kg of this extract. Similar effect was also observed with the same dose of *C. yanhusuo*.  
(Original article on page 675)

### Studies on Anti-Platelet Aggregation of *Allitridi* in Hypercholesterolemic Rabbits

Zhang Shengle (张胜乐), Xu Yingjie (徐英杰), Lu Tong (陆 通), et al

Department of Biochemistry, Shandong Academy of Medical Science, Jinan

Thirty-two New Zealand rabbits weighing 1.5~2.5 kg were divided into three groups: group I (the control group,  $n=10$ ); group II ( $n=11$ ) and group III ( $n=11$ ). They were all fed a common-stock diet. Except for the control group, group II received cholesterol (0.5 g/kg body weight) daily, while group III was fed 0.5 g/kg of cholesterol plus the *Allitridi* (diallyl trisulfide, 20 mg/kg). Blood cholesterol, platelet aggregation, platelet cAMP and cGMP index were determined by the usual techniques at fixed time. The observation was finished at the end of the 15th week. The rise in serum cholesterol was significantly reduced by *Allitridi* during the 15 weeks period of study. Platelet aggregation in group II was significantly higher than that in group I ( $P<0.05$ ), while in group III, it was close to the control group but significantly lower than that in group II ( $P<0.01$ ). There was a negative correlation between platelet cAMP and platelet aggregation ( $r=-0.42$ ,  $P<0.05$ ). Platelet cAMP in group III was little higher than that in the control group ( $P>0.05$ ), but significantly higher than that in group II ( $P<0.001$ ). There was no difference of platelet cGMP between group II and group III. It is suggested that *Allitridi* has a powerful anti-aggregating effect on platelet in hypercholesterolemic rabbits and its inhibition of platelet aggregation is mediated by an increase of platelet cAMP.  
(Original article on page 677)

### Cardiovascular Effects of Anisodamine During Endotoxin Shock in Anaesthetized Dogs

Yao Tai (姚 泰), Xiao Yongfu (肖永福), et al

Dept. of Physiology and Dept. of Infectious Diseases, Shanghai Medical University, Shanghai

Experiments were carried out on mongrel dogs anaesthetized with pentobarbital. *Escherichia coli* endotoxin (5 mg/kg) was given intravenously to produce circulatory shock. In 6 dogs, anisodamine (5 mg/kg) infused intravenously over a period of 60 min. caused increase in heart rate, left ventricular systolic pressure, positive and negative maximum dp/dt and mean arterial pressure. Renal blood flow and urinary outflow also raised. No such changes were observed in the control group of 6 animals which received saline infusion only. The results obtained indicate that anisodamine given at early stage of endotoxin shock improves myocardial contractility.  
(Original article on page 679)