

## • 实验研究 •

# 气血注射液对心肌耗氧量和红细胞中 2,3-DPG 含量的影响

北京中医学院中心实验室、生物化学研究室 张晓榕 刘春梅 陈文为

**内容提要** 气血注射液是根据益气活血治则拟定的复方注射液。通过基础实验证实：该制剂能降低豚鼠心肌匀浆耗氧量，使合成ATP的能量部分以“热”的形式温煦周身和增多红细胞中 2,3-DPG 水平，以改善其输氧功能，从而缓解冠心病患者因缺氧引起的症状。

按中医气血理论，根据益气活血治则，由人参、黄芪、当归组成复方，并制成气血注射液。临床和基础实验观察表明：该方有明显的调整血液流变性以及外周循环，缓解心绞痛症状，改善心电图缺血型ST段变化，提高运动耐受力及增强左心室功能等作用<sup>〔1〕</sup>。本文在上述基础上，进一步观察气血注射液对豚鼠心肌耗氧量，大鼠肝线粒体氧化磷酸化以及人红细胞中2,3-二磷酸甘油酸(2,3-DPG)水平的影响，从能量代谢方面，阐明该复方的生化作用原理。

## 材料和方法

### 一、试剂和药物

1. 试剂：2-[4-(2-羟乙基)-1-哌嗪]-乙烷磺酸(HEPES)为E. Merck厂出品，三羟甲基氨基甲烷(Tris)为Fluka厂出品，2,3-DPG测定采用Sigma药盒。实验中其它试剂均为分析纯。

2. 药物：气血注射液(批号8201117, 8111110)由本院中药系制剂教研室提供(每100ml气血注射液含黄芪50g，当归30g，人参10g)。

### 二、豚鼠心肌匀浆的制备

选用成年豚鼠(体重300~500g)断头处死，迅速取出心脏，去除脂肪、血管组织，按1g心肌/4ml介质液(0.25M蔗糖，0.5mM EDTA，3mM HEPES，pH7.0)，0℃冰浴下匀浆，纱布过滤，滤液为实验用心肌匀浆。用双缩脲法测定匀浆滤液蛋白含量，以牛血清白蛋白为标准品。该匀浆保存在0~4℃。

### 三、大鼠肝线粒体的制备

按常规法提取大鼠肝线粒体<sup>〔2〕</sup>。肝提取液与心肌匀浆相同，线粒体经两次洗涤，配成含蛋白10~15mg/ml的线粒体悬液。蛋白定量同上。悬浮液保存在0~4℃备用。

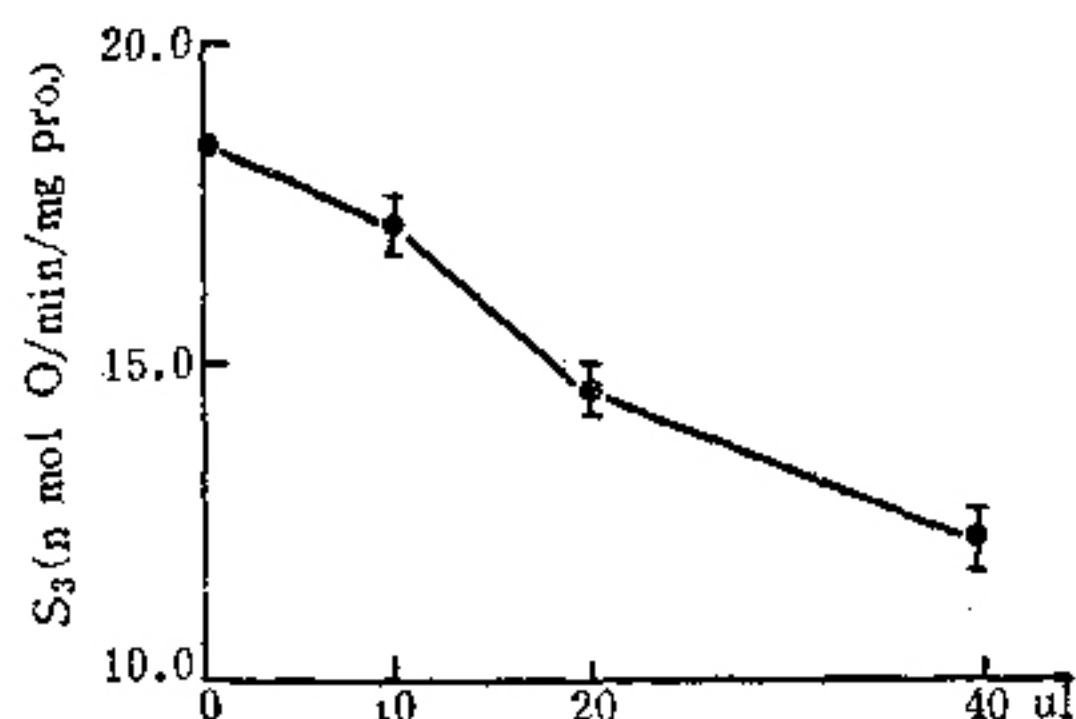
四、豚鼠心肌耗氧量( $S_0$ )，鼠肝线粒体耗氧量( $S_0$ )，呼吸控制比率(RCR)及氧化磷酸化效率(P/O)的测定。应用Clark氧电极极谱法测氧原理<sup>〔3〕</sup>，在30℃恒温条件下测定 $S_0$ 、RCR和P/O。反应基质液为0.225M蔗糖，1mM EDTA，5mM  $MgCl_2$ ，15mM KCl，15mM  $Na_2H_2PO_4$ ，50mM Tris-HCl，5mM谷氨酸，10mM DL-苹果酸，pH7.5的缓冲液。取1.8ml基质液，分别加入0.2ml心肌制剂(约含4mg蛋白左右)，线粒体悬浮液(约2mg蛋白左右)，先记录2分钟的耗氧(内源性耗氧)后，加入100mM ADP 5  $\mu$ l，激活耗氧。呼吸状态3( $S_3$ )为加入ADP后耗氧量，呼吸状态4( $S_4$ )为ADP转为ATP后的耗氧量；呼吸控制比率(RCR)为 $S_3/S_4$ 比值，反映线粒体功能；氧化磷酸化效率(P/O)，当底物存在时，加入ADP后通过氧化磷酸化反应合成ATP所消耗的微克原子氧的比值，理论应为3.0<sup>〔4〕</sup>。

### 五、2,3-DPG的测定

取8份3.5ml健康人红细胞(压积为40~50%)分装8个50ml三角瓶中，分为四组(每组两份)。除对照组外，其它3组，分别加入0.2、0.5和1.0ml气血注射液。用Krebs' S缓冲液(0.113M NaCl，4.9mM KCl，1.24mM  $MgSO_4$ 葡萄糖1.0g/500ml，16mM  $Na_2HPO_4$ ，pH 7.4)补足至终体积4.5ml。37℃震荡温育4~6小时后，各取1ml温培后红细胞于1ml三氯醋酸(12%)中，混匀，离心，取上清液，按Sigma Technical Bulletin No 665操作，测定2,3-DPG含量(nmol/ml RBC)<sup>〔5〕</sup>。

## 结 果

一、气血注射液对豚鼠心肌耗氧量( $S_0$ )的影响，曾有文献指出<sup>〔1〕</sup>，该注射剂可降低心脏耗氧作用。为证实这种现象，直接测定心肌匀浆耗氧量，如附图。



附图 气血注射液对豚鼠心肌耗氧量的影响。数值为6次实验平均值

可见气血注射液明显降低心肌耗氧量,并呈量效关系。当加入40 $\mu$ l药物时抑制率为39.8%。由于心肌组织匀浆中含有丰富的线粒体,它是心脏耗氧量最多的亚细胞器,这可进一步证明气血注射液经降低耗氧量途径达到减轻心肌负荷,缓解由于缺氧引起的不良反应,达到保护心肌正常功能的作用。

二、气血注射液对大鼠肝线粒体 $S_3$ , RCR和P/O的影响: 气血注射液降低大鼠肝线粒体的 $S_3$ ,见表1, 10 $\mu$ l时抑制率达55.5%; 气血注射液可降低氧化磷酸化效率,并有解偶联作用,且作用呈量效关系。

表1 气血注射液对大鼠肝线粒体氧化磷酸化的影响

气血注射液 加入量( $\mu$ l)	M $\pm$ SD		
	$S_3^*$	RCR	P/O
空白对照	58.7 $\pm$ 0.6	5.92 $\pm$ 0.83	2.39 $\pm$ 0.01
5	35.4 $\pm$ 1.3	2.80 $\pm$ 0.18	2.00 $\pm$ 0.08
10	26.2 $\pm$ 1.6	2.45 $\pm$ 0.07	1.79 $\pm$ 0.25

注: 数值为三次实验的平均值

\* $S_3$ 单位为 nmol O/min/mg蛋白

已知氧的消耗与能量的生成是紧密相关的,从气血注射液对 $S_3$ ,  $S_4$ 和RCR的变化可以反映该制剂减少ADP磷酸化偶联生成ATP,即解偶联作用,使其生成的能量以“热”的形式出现。从整体考虑,上述现象是调节机体能量利用的一种重要方式。

三、气血注射液对人红细胞2,3-DPG含量的影响: 本实验观察到气血注射液也可增加健康人红细胞2,3-DPG水平(表2)。其作用有明显的量效关系,说明该注射液有增多红细胞中2,3-DPG水平作用。由于2,3-DPG具有降低氧合血红蛋白( $Hb(O_2)_4$ )的结合力,这有助于周身组织细胞获得更多的氧。

表2 气血注射液对人红细胞2,3-DPG含量的影响

气血注射液 加入量(ml)	2,3-DPG 含量 <sup>*</sup> M $\pm$ SD
空白对照	0.547 $\pm$ 0.274
0.2	0.617 $\pm$ 0.292
0.5	1.075 $\pm$ 0.607
1.0	1.819 $\pm$ 0.719**

注: 数值为四次实验平均值; \*2,3-DPG含量单位为 nmol/ml RBC; \*\*与对照比较,  $P < 0.05$

## 讨 论

气血注射液可降低豚鼠心肌耗氧量,这种现象可对心脏起保护作用。冠心病患者常由于供血不足,氧供需平衡失调,引起心脏功能紊乱。药物治疗,可降低心肌耗氧量,减轻心脏负担,调整氧供需平衡,从而缓解心绞痛等症状。

气血注射液同样降低大鼠肝线粒体耗氧量,并有解偶联作用,即降低氧化磷酸化效率,所产生的能量以热的形式出现,这可调节四肢体温,改善外周循环,有助于“温煦四肢”的作用,也符合益气活血治则。

此外,气血注射液使人红细胞2,3-DPG含量明显增多,促使血红蛋白在同样的氧分压下向组织释放更多的氧,以弥补缺氧,改善红细胞输入氧功能,这对心绞痛、心肌梗塞、心衰、休克等疾病都有治疗意义。

以上结果说明气血注射液通过益气活血治则治疗心气虚患者的生化药理基础。

## 参 考 文 献

1. 陈俊杰,等。益气活血法治疗冠心病心绞痛的临床及实验研究。中西医结合杂志 1985; 11(5): 658.
2. Adelman HC, et al. Dietary and hormonal effects on adenosine triphosphate: adenosine, monophosphate phosphotransferase activity in rat liver. J Biol Chem 1968; 243:2538.
3. Lessier MA. Oxygen electromeasurements in biochemistry analysis. Methods of Biochemical Analysis 1969; 17:1.
4. 邱洪法,等。气血实质的探讨: 四种中药和生脉液对鼠肝线粒体(体外)氧化磷酸化的影响。中西医结合杂志 1981; 1(1): 32.
5. The ultraviolet enzymatic determination of 2,3-diphosphoglyceric acid(2,3-DPG). Sigma Technical Bulletin, No35-UV, 1980.

### Effects of Qixue(气血) Injection on Heart Oxygen Consumption and 2,3-DPG in RBC

Zhang Xiaorong (张晓榕), Liu Chunmei (刘春梅), Chen Wenwei (陈文为)

Dept. of Biochemistry, Central Laboratory, Beijing College of TCM, Beijing

Qixue injection (QXI), consisting of *Panax Ginseng*, *Astragalus membranaceus* and *Angelica sinensis*, is a preparation for the treatment of coronary heart disease (CHD) according to the principle of "replenish the Qi(气) and promote the blood circulation". The experimental results showed: (1) The QXI could lower the oxygen consumption of guinea pig heart homogenate, (2) it was an uncoupling agent which could transform the ATP which was converted to "Heat" for warming the peripheral tissues and (3) it could also increase the formation of 2, 3-DPG in healthy human RBC (in vitro) and there by improve their oxygen supply. The above-mentioned three pathways might account for the remission of the anoxia-induced symptoms of CHD patients.

(Original article on page 606)

### Dihydroxyacetophenone and Dihydroxyphenyllactic Acid Increased Blood Flow in Rat Liver Microcirculation

Zhang Qingbo (张清波), A. Koo (顾克仁)\*, et al

Dept. of Infectious Disease, Huashan Hospital, Shanghai Medical University, Shanghai

\* The Chinese University of Hong Kong, Hong Kong

3, 4-Dihydroxyacetophenone (DHAP) and 3, 4-Dihydroxyphenyllactic acid (DHPLA), the natural compound from the Chinese medicinal herbs *Ilex pubescens* and *Salvia miltiorrhiza*, were synthesized for the study of their vascular and choleretic effects. Blood flow on the liver surface was monitored by a Laser-Doppler flowmeter, control flow value was taken as 100%. Bile secretion rate was measured with a drop counter from a cannula in the common bile duct. Results showed that both drugs significantly increased hepatic blood flow and bile secretion rate in dose-dependent manner, the DHPLA was more potent than DHAP in enhancing hepatic blood flow, while the DHAP was more potent than DHPLA in enhancing choleretic action.

(Original article on page 608)

### Memory Facilitation Induced by *Panax Ginseng* and *Pseudoginseng* in Mice

Zhang Lei (张磊), Zhang Juntian\* (张均田)

Institute of Clinical Medicine, The China-Japan Friendship Hospital, Beijing

\* Dept. of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing

*Panax ginseng* has been used as a tonic in TCM for several thousand years. Recent pharmacological studies suggested that *ginseng* has various effects, e. g., the stimulation or inhibition of the central nervous system, the regulation of endocrine function and the carbohydrate, fat and protein metabolism, the anti-fatigue as well as memory-enhancing effects, etc. On the basis of these researches, studies of *ginseng* on learning and memory were carried out. Because *Panax pseudoginseng* is similar to *ginseng* in many chemical components, a comparison of the pharmacological effects between these two drugs was also made. 20% alcohol extracts of *ginseng* and *pseudoginseng* roots were used in mice for facilitation of memory using one trial passive avoidance responses and water maze methods. The results indicated that *ginseng* improved impairment of acquisition of memory produced by anisodine and pentobarbital, and disruption of consolidation of memory caused by cycloheximide and sodium nitrite, and it was also capable of antagonizing alcohol-induced deficit of retrieval. Similar effects were observed with *pseudoginseng* in the case of impairments of acquisition of memory caused by sodium nitrite and anisodine. It is obvious that *ginseng* is more effective than *pseudoginseng* on learning and memory in mice. The study on the mechanism of action of *ginseng* and *pseudoginseng* in promoting learning and memory is being carried on.

(Original article on page 610)