·临床论著·

冠心病血瘀证患者血小板超微结构和功能的研究

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内容提要 在电镜下对冠心病血瘀证患者的血小板作超微结构和功能的多项指标研究结果：患者大型血小板比例增高，易于变形，糖萼增厚，膜的异常运动增多，糖原增多，对ADP敏感；聚集功能增强，细胞膜融合发生较早，释放反应活跃，吞噬能力降低，腺苷酸环化酶活性降低；对钙的反应增强；丙二醛含量增高。这些异常表现与血瘀程度基本呈一致关系，故认为血小板的变化是冠心病血瘀证中的一种实质性表现，与血小板形态、功能密切相关。

冠心病血瘀证及血瘀证与血小板的关系，是当前中西医结合领域充分重视并亟待深入研究的课题，但从血小板形态与功能相结合角度所做的研究尚不多见。自1986年以来，我们做了健康人及冠心病血瘀证患者血小板超微结构及功能的研究，并对其机理进行了探讨。

资料与方法

一、观察对象：选择无血液病、冠心病等重要疾病，无血瘀表现的健康人36名作对照组（Ⅰ组），年龄34～72岁，平均51.9岁；其中男24例，女12例。冠心病血瘀证患者64例（Ⅱ组），年龄39～70岁，平均62.2岁；其中男49例，女15例。稳定型心绞痛8例，不稳定型心绞痛8例，陈旧性心肌梗塞20例，急性心肌梗塞28例。冠心病的诊断按国际心脏病学会和协会及世界卫生组织1979年的命名及诊断标准[1]进行，血瘀证的诊断主要依据第二届全国活血化瘀学术会议修订的“血瘀证诊断标准”[2]，并结合冠心病血瘀证的临床特点，进行血瘀程度的评分，然后将患者分为轻度血瘀（Ⅱa组）11例，中度血瘀（Ⅱb组）18例，重度血瘀（Ⅱc组）35例。

二、实验方法：观察对象停服各种对血小板功能有影响的中西药物，并戒烟酒两周以上，取空腹静脉血制成富含血小板血浆(PRP)，然后分别进行以下制备。(1)基本制备方法：取PRP0.5ml，离心后用缓冲的戊二醛4°C下固定2小时，漂洗、锇酸后固定，梯度乙醇脱水，包埋液浸泡，聚合，LKB Ⅱ型超薄切片机切片，日本JEM-100CX电镜(80kv)作血小板一般形态的观察。依观察项目的不同，加入不同试剂。(2)取PRP0.5ml，加入含硝酸镧的固定液，以作细胞膜结构的观察。(3)取PRP0.5ml，加入20%乳胶（自制，直径0.45μ）0.1ml，60分钟后固定，作吞噬能力的观察。(4)取PRP0.9ml，加入2×10^{-4}M的ADP (New York, Serva生产) 0.1ml，15分钟后再行固定。(5)取PRP 1ml，离心，固定后用0.1M二甲砷酸钠缓冲液4.5%葡萄糖4°C下清洗过滤，置孵育液中60分钟，再行漂洗、脱水，做腺苷酸环化酶活性观察。(6)取PRP及(4)中加入ADP作用后的PRP各一份，分别置于铺有Formar膜的铜样品托上，固定、漂洗、脱水、醋酸异戊脂置换，干燥，喷镀金膜后作扫描电镜观察。(7)取PRP0.5ml，离心，加入0.1ml 10mM N-乙基顺马来酰亚胺，水浴，加0.67%硫代巴比妥酸1ml(北京化工厂出品)，水浴后正丁醇提取，在日本津岛PF-450型荧光分光光度计上以激发波长515nM，发射波553nM读数，计算1×10^9个血小板的丙二醛含量。并以4×10^{-6}M 1，1，3,3-四乙氧基丙烷 (Flnk, AG Buchs生产) 代替PRP，操作，绘制标准曲线。

结 果

一、血小板一般形态的观察：Ⅰ组血小板多为中等大小的盘状细胞。加用硝酸镧的标本中可见到镧所示踪的细胞糖萼层。细胞浆中α颗粒，致密颗粒及线粒体分布均匀，开口管道系统(OCS)和致密管道系统(DTS)无明显扩张。微管束分布于细胞周边，糖原颗粒分散或呈小簇状散布于胞浆中(图1)。Ⅱ组血小板有以下特点：(1)细胞形态变化较多，而盘形细胞减少(图2)。扫描电镜见细胞表面皱折及伪足增多，可

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表 1　血小板形态观察统计　(M±SE)

组别	大血小板(%)	畸形血小板(%)	伪足变形血小板(%)	糖萼增厚血小板(%)	α颗粒计数	致密颗粒计数	糖原增多血小板(%)
Ⅰ	6.4±0.20	68.4±0.39	12.2±0.27△	6.7±0.21	27.4±0.12	1.72±0.005	4.2±0.17
Ⅱa	13.5±0.52	38.0±0.73	24.1±0.64	66.7±0.71	24.0±0.10	1.21±0.008	41.2±0.74*
Ⅱb	17.7±0.45	31.3±0.55	31.2±0.55△△△	80.2±0.47△	20.9±0.08	0.75±0.007	57.1±0.58*
Ⅱc	27.5±0.38	6.9±0.21	43.0±0.42△△▲	83.0±0.32▲	12.6±0.05	1.0 ±0.007	52.0±0.42*

*各Ⅱ组间对比 P>0.05；与Ⅱa 比△P<0.05，△△P<0.01，△△△P>0.05；与Ⅱb比▲P>0.05；余P均<0.01

见血小板粘附于红细胞的现象。(2)大型血小板比例增多(直径>3.5μ)。(3) 有聚集倾向的血小板增多。(4) 血小板颗粒减少，空泡增多，颗粒之间可互相融合。(5)环周微管消失并出现微丝。(6)管道系统扩张及增生。(7)易见异常颗粒，大小与α颗粒相似，有界膜包绕，内容物为胞浆、膜状碎片及类似线粒体的峭状物等。(8) 糖原、脂滴增多的细胞比例多。各组主要观察数据见表1。

二、血小板吞噬能力的观察：Ⅰ组67.7%血小板有吞噬能力，吞噬数量可达几十个乳胶颗粒(图3)。经OCS在细胞膜上的开口或经细胞膜下陷而吞入。Ⅰ组血小板的吞噬率：Ⅱa组40.3%，Ⅱb组32.9%，Ⅱc组10.7%，均较对照组明显降低(图4)。吞入的乳胶数量也很少，多为1～3个。其中能吞噬的细胞多为中型血小板，而大型及糖原含量多的细胞几不吞噬，但常见颗粒粘附于细胞膜外。同时细胞变形较明显，血小板颗粒减少并有聚集倾向。

三、血小板聚集形态的观察：加入ADP后各组形成聚集体比例：Ⅰ组60.4%，Ⅱa组81.1%，Ⅱb组77.6%，Ⅱc组89.7%。Ⅰ组与各Ⅱ组间P<0.01。Ⅰ组聚集体中细胞间隙较清楚，并可见镉颗粒附着在两侧细胞膜上，少数间隙中形成细丝样桥状联系。Ⅱ组聚集体变大，多数细胞间隙中有桥状联系而模糊不清，并随血瘀加重，更易见到细胞间隙消失，细胞膜融合。聚集过程中细胞间隙变窄时糖萼呈现单层排列，当细胞膜融合时镉颗粒仅存在于聚集体周边及OCS中(图5)。当两个细胞相距较远时，即可见相互的细胞膜已发生作用而变得模糊不清。血小板颗粒减少较明显并可见到往细胞外释放颗粒。扫描电镜下见伪足增多增长并肿胀增粗，相互缠绕，细胞间界限不清，形成大的实体性聚集团块。

四、血小板对钙的反应的观察：加入钙后，各组中大多数细胞都发生聚集；形成大的聚集体，聚集程度亦较加入ADP后为强。胞浆中有密集的钙的细小点粒。Ⅰ组约1/3细胞中尚保留一定数量的血小板颗粒，而Ⅱ组细胞内颗粒基本上全部排空，随着血瘀程度加重，细胞膜融合程度及钙粒更密集的细胞都增多，以Ⅱc组最为多见。释放反应亢进的现象更易见到。如：血小板颗粒移于细胞膜下，经膜释放，颗粒膜互融使颗粒物质溶于胞浆，胞浆物质经OCS或细胞膜排出，胞浆或代谢物质释出等(图6)。

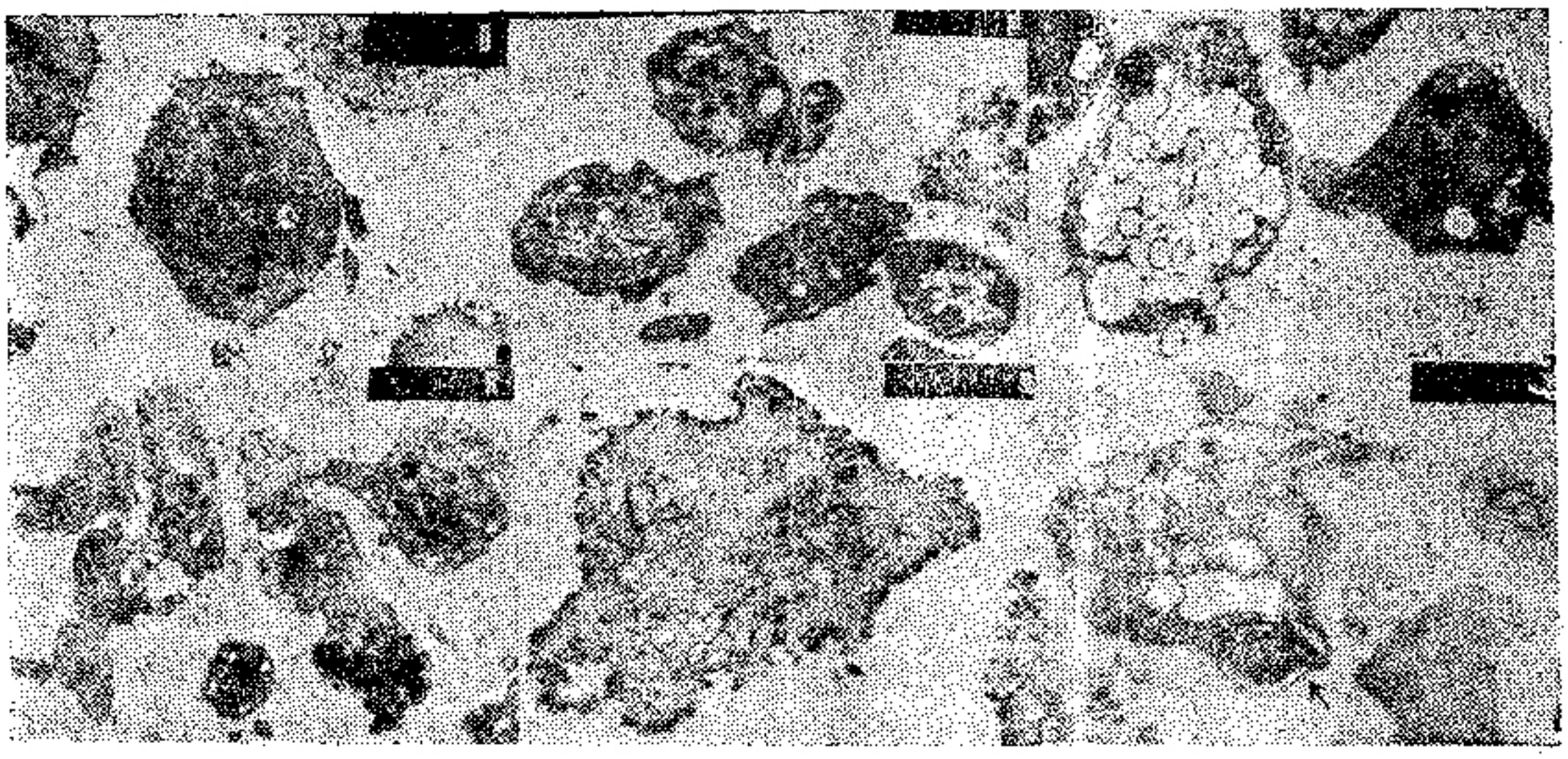
五、血小板腺苷酸环化酶活性观察：Ⅰ组细胞膜上及线粒体膜、颗粒膜、OCS膜上均可见有明显的酶着色。Ⅱa及Ⅱb组可见部分细胞膜上有轻度的酶着色，Ⅱc组则很少见有着色的细胞。随血瘀加重而聚集倾向加重，此时细胞膜相互作用及变得模糊的地方均不见有酶的着色。

六、血小板丙二醛(MDA)含量测定：测定结果：Ⅰ组血小板内MDA含量为7.92±2.54nM/1×10⁹个血小板，Ⅱ组血小板内MDA含量(nM/1×10⁹个血小板)分别为Ⅱa组11.02±3.54，Ⅱb组10.56±3.47，Ⅱc组11.48±3.40。Ⅰ组与各Ⅱ组间P<0.05。

讨　论

一、患者血小板易于激活，发生聚集，是“内结为瘀”的形态基础。观察所见患者血小板易于变形、聚集，膜的运动、融合性强，对ADP、钙反应性增强及释放反应活跃等，使得循环血液中血小板聚集体增多，且由于膜易于融合而成为不可逆性聚集。聚集体粘附血液其它成分，不断增大，附着于血管壁，使血管腔逐渐变窄，血流缓慢淤滞，造成“血脉凝泣”、“血行失度”、“心脉瘀阻”，而出现胸闷、胸痛，唇暗，舌青紫，脉涩或结、代等症候。这些血瘀表现是血小板功能异常所致的病变的结果，且又可进一步致病，病情不断加重。血小板异常激活后的作用，恰与血瘀一样是血行不畅造成的结果，又可致气血、阴阳进一步失调而加重瘀阻。

二、血小板在冠心病血瘀证中的异常形态构成“污秽之血”。研究中所见患者组血小板异常表现，如糖萼增厚，吞噬力下降，含异常颗粒或糖原增多的血小板等，是造成血瘀的原因之一。血瘀日久，损伤脉络，即会构成“离经”的“污秽之血”。所见血小板各种



图序从左至右上行1、2、3，下行4、5、6。图1 I组，正常血小板。细胞膜外侧所附黑色点粒为示踪剂镧显示的糖萼。细胞内结构可见血小板颗粒，线粒体，糖原，环周微管，开口及致密管道系统。×13 000 图2 IIc组，细胞变形明显，血小板颗粒减少，OCS扩张，增生。×6 600 图3 I组，血小板内充满了吞入的乳胶颗粒。×13 000 图4 IIc，血小板不吞噬乳胶颗粒，细胞变形明显，颗粒减少，糖原增多，有聚集倾向，细胞膜上有乳胶颗粒粘附。×8 300 图5 IIc组，血小板聚集体中细胞膜融合，糖萼消失。聚集体周边糖萼存在。与图1比较，糖萼明显增厚。×10 000 图6 IIb血小板伪足形成。细胞内颗粒减少，管道扩张，胞浆内充满细小的黑色钙粒，代谢产物浓缩聚集，向细胞外释出(↑)。×16 000

异常超微结构，使"污秽之血"有了血液形态学方面的证实。所以，改善血小板形态和功能，也是当前防治冠心病的一条重要途径。

三、从冠心病血瘀证患者血小板功能失调看本虚标实问题。冠心病是以血瘀为主证的疾病，从标本辨证而论，其基本病机为本虚标实。标实可指血实，为各种有形之"污血"及血小板异常的功能亢进表现。本虚则指反应血小板本身"正气"不足的表现。将本虚标实证从血小板超微结构与功能的角度联系起来，有助于对这一问题的理解及进一步的探讨，见表2。

表2 本虚标实证微观分析

	超微结构表现	功能异常	病变结果	治则
本虚	细胞内糖原大量堆积 细胞内乳胶颗粒很少 无明显AC着色	能量代谢障碍 吞噬能力降低 AC活性降低	正气虚， 生理功能 降低	补不足 损有余 以通为
标实	伪足变形等细胞增多 血小板颗粒减少 糖萼增厚 聚集体形成，增大	膜运动转化增多 释放反应亢进 细胞膜易于融合 对致聚剂敏感 MDA产量增多	邪气盛， 形成"离 经之血"、 "污秽之 血"	补，活 血化瘀

四、血小板在血瘀证中的异常表现，为异病同治的病理生理学基础。血小板是多功能细胞，其作用涉及人体各个系统，而血瘀证也是临床多种疾病的共同证候表现。血小板与血瘀证作用于人体生理病理过程的广泛性，展示出二者之间有着内在的联系。冠心病中的多数症状是血瘀证中的共同表现。冠心病的临床分型及病理变化不完全相同，但血小板异常改变却与血瘀程度基本是一致的关系，说明了血小板在血瘀证中所起的作用。故血小板的超微结构及功能变化，可作为各种血瘀证疾病中纵的联系，成为中医学术中异病同治法及活血化瘀治法理论基础的一个重要方面。

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A Study on Platelet's Ultrastructure and Function of Coronary Heart Disease Patients with Blood-Stasis Symptom-Complex

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36 healthy persons (as control group) and coronary heart disease (CHD, 8 stable angina pectoris, 8 unstable angina pectoris, 20 OMI, 28 AMI) with three different degrees of blood-stasis, patients' platelets were studied ultrastructurally and functionally on many items. Under transmission electron microscope (TEM) it were found that the proportions of large platelet were 6.4±0.20 in group I (control group), 13.5±0.52 in group IIa (CHD with mild blood-stasis), 17.7±0.45 in group IIb (CHD with moderate blood-stasis), and 27.5±0.38 in group IIc (CHD with severe blood-stasis); platelet with thicker glycocalyx in each group were 6.7±0.21, 66.7±0.71, 80.2±0.47 and 83.3±0.32 respectively; the latex particle phagocytic rate by platelets were 67.7±0.39, 40.3±0.58, 32.9±0.71 and 10.7±0.26 respectively; platelets with increased glycogen particles were 4.2±0.17, 41.2±0.74, 57.1±0.53 and 52.0±0.42 respectively. Between groups, the most figures presented above had statistic differences ($P<0.05$, $P<0.01$). TEM and SEM observation showed that in patients group, platelet was easier to change its shape, to aggregate and adhere, was more sensitive to ADP, more mutual transformation in intracellular membrane system, and had increased ability to uptake calcium ion and respond to it. Fusion of platelet memberane in aggregating was earlier, release reaction was more active which caused reducing the intracellular granule. TEM histochemistry study and malonaldehyde (MDA) estimation demonstrated platelet adenyl cyclase (CA) activity decreased and MDA content increased. The study showed that the more patients' platelets were in activated state, the lower were some of their functions (such as phagocytosis and CA activity). These abnormality related to the severity of blood-stasis but did not to the type of CHD. The result showed that blood-stasis was one of the essences of CHD. The morphological and functional abnormality of platelet were some of the basis of blood stasis symptom-complex. (Original article on page 593)

Clinical and Experimental Study on Effect of *Salvia miltiorrhiza* on Microcirculation and 2, 3 DPG of Coronary Heart Disease Patients

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Microcirculatory flow of nail bed, blood 2, 3 DPG and deformability of RBC in 40 cases with different degree of CHD were studied and found that: (1) velocity of flow was inversely proportional to vessels involved ($P<0.01$); (2) *Salvia miltiorrhiza* (SM) increased velocity of flow, with its action delayed in proportion to vessels involved; (3) 2, 3 DPG of RBC and blood were increased after SM administration; (4) deformability and filtration of RBC were increased after SM administration ($P<0.01$). In 12 rabbits, 20% dextrane was given to form acute microcirculatory disturbance model. Mesenteric microcirculatory flow, blood 2, 3 DPG, and deformability and filtration time of RBC were studied before and after dextrane and SM administration. It was found that: (1) velocity of blood flow was slowed after dextrane and increased after SM ($P<0.01$); (2) blood and RBC 2, 3 DPG were decreased after dextrane and increased by SM ($P<0.01$); (3) deformability and filtration time were decreased after dextrane and increased after SM ($P<0.01$). The blood gases and 2, 3 DPG of 12 rats were studied after putting into an airtight bottle containing 12~15% O_2 for 30 min. to form acute hypoxia model. It was found that PO_2 and 2, 3 DPG were lowered in control and only slightly lowered in SM group ($P<0.01$). It was concluded that there was microcirculatory and 2, 3 DPG disturbance in patients with CHD which could be improved by SM. The increase of microcirculatory flow after SM was related to increase of 2, 3 DPG of RBC and deformability of it, which maintained a relative high PO_2 of blood after an hypoxic condition. (Original article on page 596)

Clinical and Hemorrheologic Study in Treating Blood-Stasis Syndrome of Angina Pectoris with Huoxue(活血) Granule

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This paper reports treating blood-stasis syndrome of coronary heart disease(CHD) angina pectoris with Huoxue granule(HXG) and its effect on hemorrheology. 50 cases were divided into two groups, one group was treated with HXG, another with placebo (single blind). In HXG group the total effective rate for angina pectoris was 86.7%, marked effective rate 33.3%, effective rate for ischemic ECG changes was 40%. All of these were better than placebo group statistically ($P<0.025$, 0.005 0.025). Experimental results showed that HXG was able to inhibit platelet adherence and aggregation, decrease whole blood viscosity in low shear, prolong reacting time and coagulating time of thrombolastrogram, improve erythroid deformability. So the mechanism for HXG in treating angina pectoris may be preventing coronary artery injury caused by platelet adherence, aggregation, release and hypercoagulation, improving microcirculation and myocardial blood supply. These results suggest that HXG is an effective antianginal medicine. (Original article on page 599)