

The function of microparticles and the research progress of diabetes and microparticles

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Received: Sep 29, 2015

Accepted: Oct 21, 2015

Published: May 03, 2017

DOI:10.14725/gjems.v3n1.a1332 **URL:**<http://dx.doi.org/10.14725/gjems.v3n1.a1332>

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Abstract

MPs are vesicles released by cells when stimulated by physical (e.g. shear force) or chemical (e.g. agonists) factors, as well as cells undergoing apoptosis or exposed to inflammatory conditions. MPs are 100~1000 nm in diameter, have membrane cytoskeletons, express phosphatidylserine (PS) on the surface, and lack of nuclei. Surface molecules, enzymes, RNA and DNA are conveyed via MPs from origin cells to target cells. As mediators of information transfer, MPs have been proposed to impose pro-inflammatory and pro-coagulant effects in many disease states, such as cancer, venous thromboembolism, arteriosclerosis, and diabetes mellitus. The hypercoagulable state associated with diabetes is well recognized. More T2DM patients have died from thrombotic diseases. The endothelium-derived MPs in diabetic patients were elevated. TF-positive MPs concentration was increased and procoagulant activity of MPs was elevated. It is worth to research the role of MPs in the hypercoagulable state of diabetic patients.

Key Words

Microparticles; Tissue factor; Diabetes; Hypercoagulable

微粒的功能及其与糖尿病的研究进展

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【摘要】微粒是由细胞受物理或化学刺激或者凋亡时产生的带细胞膜的囊泡。其大小在 100~1000nm 之间, 表面的膜细胞骨架上含有磷脂酰丝氨酸及多种细胞分子, 其包膜内无核, 含有细胞分子、RNA、DNA 等。在机体中起到信号传递的作用。近年来发现, 微粒参与多种疾病的病理生理过程, 如糖尿病、冠心病、深静脉血栓、肿瘤等, 在以上疾病过程中, 微粒主要有促炎、促凝等作用。糖尿病患者存在明显的高凝状态, 糖尿病患者合并心血管疾病死亡率近年来有增高趋势, 血栓事件较非糖尿病患者增加。糖尿病患者血浆中内皮源性微粒数目增多, 组织因子阳性微粒比例升高, 微粒促凝活性升高。微粒的改变是否参与糖尿病患者的高凝状态的发生值得研究。

【关键词】微粒; 组织因子; 糖尿病; 高凝状态

前言

微粒(microparticles, MPs)是一类由细胞在受到应激、刺激因子刺激或者凋亡时产生的细胞囊泡^[1]。其大小在 100~1000nm 之间, 含有膜细胞骨架, 表面含有一定量的磷脂酰丝氨酸(phosphatidylserine, PS), 无核^[2]。自 1967 年 Wolf^[3]首次报道血小板微粒以来, 人们对其进行了大量的研究。目前发现, 几乎所有细胞

均能释放微粒,例如:内皮细胞、白细胞、红细胞、单核细胞、血小板、平滑肌细胞等^[4-5]。微粒表面常表达各种表面分子,这些表面分子与相应受体结合从而激活目标细胞。微粒内含有多种细胞因子、酶、RNA、miRNA、甚至是DNA^[6-7],通过释放其内容物激活目标细胞、改变细胞基因型或者表型。由此看来,微粒在体内起到传递信息的作用。在健康个体,血浆微粒的浓度维持在一个较低的水平,甚至很难检测,在疾病状态下,其浓度变化较大^[8]。目前发现在疾病状态下,微粒有明显的促炎、促凝作用,本文就微粒在凝血过程中的作用的研究进展作一概述。

1 MP的形成与释放

物理的(如剪切力)或者化学的(如激动剂)细胞刺激因素、凋亡,均能刺激微粒的形成。常见的激动剂有:低密度脂蛋白(low-density lipoprotein, LDL)、凝血酶、促炎症性细胞因子如TNF- α 、IL-1B等^[9]。虽然目前关于MPs的形成和释放的机制尚不明了,但可以明确的是,细胞骨架的重组和磷脂酰丝氨酸的外化是MPs形成过程中的关键两步。正常情况下,细胞膜上的磷脂呈不对称性分布,磷脂酰丝氨酸主要分布在细胞膜内侧。细胞被激活时,局部的质膜破裂,局部流体静水压变化导致质膜起泡,继而收缩的肌球蛋白系统促使小泡脱落^[10]。在这一过程中,钙离子的内流是决定性步骤^[11]:钙内流激活蛋白酶,使局部细胞骨架蛋白(包括肌动蛋白、膜收缩蛋白)重组、分解,导致质膜的突起从皮层激动蛋白脱落,即引起微粒的释放。

2 MP的促凝活性

在正常状态下,血液循环中存在少量的微粒。其中,血小板源性微粒能产生保护性的低浓度促凝活性^[8]。在疾病状态下,微粒的促凝活性有明显升高。从微粒的结构、成分来看,其表面表达的PS和组织因子(Tissue Factor, TF)是其促凝活性的重要原因。

带负电荷的PS能为带有阳离子域的凝血蛋白、凝血因子、凝血酶的反应和生成提供催化表面^[12]。同时,PS能增加TF凝血活性。PS与TF同时表达在微粒的外膜时,微粒的促凝活性最强^[13],并且被认为是凝血级联反应的引发剂^[14-15]。TF是FVII/VIIa受体,TF与FVII或FVIIa结合而形成具有蛋白酶活性的TF:FVIIa复合物,从而活化FX和FIX来启动凝血过程^[16]。既往人们认为,TF只存在于血管周围的组织细胞中,当血管内皮受损,组织细胞暴露时,其上表达的TF与血液中的FVII接触从而触发凝血反应。但是1999年,研究者发现,健康个体的血液中存在很低水平的功能性TF,即所谓的血源性TF,在体外模型中,这些血源性TF参与了血栓形成^[17]。目前有一部分学者认为,微粒表面表达的TF,除了能触发凝血级联反应,还能对凝血块的增长起到促进作用。血管损伤时,血管壁的TF能促发凝血,但随即被血小板、血凝块包裹,从而阻止它进一步参与凝血。循环中的TF则能持续存在并发挥作用,黏附在血凝块并促进其继续生长^[12]。有动物实验发现,白细胞源性TF+微粒能促进小鼠提睾肌动物血栓的增长^[18]。目前报道表达TF的微粒包括:单核-巨噬细胞源性微粒,白细胞源性微粒,内皮细胞源性微粒。而血小板微粒是否表达TF尚有争论^[12, 19]。

2.1 血小板源性微粒 血液中的MPs 70%~90%来自于血小板^[20]。血小板源性微粒(PMPs)表面含有FVIII受体^[21]和FVa受体^[22],以及高/低亲和力的FIXa结合位点^[23]。FVIII是FIXa/VIIIa的辅助因子;FVa能与FIXa结合形成促凝血酶复合物。血小板源性微粒上的这些受体与相应的凝血因子结合,从而促进凝血级联反应。这些发现也表明,PMPs与局部激活的血小板相比,能够发挥更长距离及更长时间的促凝活性。有研究表明,PMPs的促凝活性比活化的血小板高50~100倍^[24]。

2.2 单核细胞源性微粒 单核细胞源性微粒(Monocytes-derived MPs, MDMPs)是表达TF的主要微粒。在健康个体中,血源性TF 95%表达在外周血单核细胞上,5%表达在MPs上^[25]。组织因子表达阳性(TF+)

的单核细胞能释放 TF+ 的微粒^[26]。MDMPs 具有很高的促凝活性, 因其表面除了表达 PS 及 TF, 还表达 PSGL-1 (P-selectin glycoprotein ligand -1), 其能够通过结合 P 选择素与激活的血小板、内皮细胞结合, 同时起到累积 TF 的作用^[27]。

2.3 内皮源性微粒 内皮源性微粒 (Endothelium-derived MPs, EMPs) 也通过多种途径参与凝血过程。许多血液呈高凝状态的疾病都发现血浆 EMPs 水平升高, 如系统性红斑狼疮^[28]、动脉粥样硬化、糖尿病^[29]、血小板减少性紫癜^[30]等。这些说明, EMPs 可能具有促凝作用。大量实验发现, TNF- α , 脂多糖, LDL 等刺激成熟的人脐静脉内皮细胞, 能导致 EMPs 表面表达 TF 增加。体外促凝试验分析发现, 这些 EMPs 能缩短血浆凝固时间。这证明了 EMPs 的促凝作用。但在去除了 FVII 的血浆中, 则没有这种作用^[31-32]。这说明, EMPs 的促凝作用是依赖于表面表达的 TF 来实现的。同时, 表面表达超大血管性血友病因子(von Willebrand factor, vWF)多聚体的 EMPs 可与血小板、PMPs 结合, 从而促进血小板聚集, 形成网状结构, 这种聚集较血液中游离的 vWF 诱导的血小板聚集更加牢固^[33-34]。再次, EMPs 表面表达的其他黏附分子, 如细胞间黏附分子 1 (intercellular adhesion molecule-1, ICAM-1)、血管细胞黏附分子 1 (vascular cell adhesion molecule-1, VCAM-1)、血小板内皮细胞黏附分子 1 (Platelet-endothelial cell adhesion molecule-1, PECAM-1)、E 选择素等, 也可进一步加固血小板与 EMPs 的连接^[35]。

3 MPs 的抗凝作用

在特定的条件下, 微粒具有抗凝作用。这是由其来源及所受的释放刺激所决定的。研究发现, 微粒表面表达的具有抗凝作用的分子包括: 血栓调节蛋白(thrombomodulin, TM)、组织因子途径抑制物(tissue factor pathway inhibitor, TFPI)、内皮细胞蛋白 C 受体 (endothelial protein C receptor, EPCR)^[36]。血液中 TF: F VIIa 复合物由 TFPI 调节, 它由内皮细胞生成并在血液中循环, 以防止不适当的凝血的发生。所以有人假设 MP 所表达的 TF 则有可能被 MP 所表达的 TFPI 所抑制。目前已经在内皮细胞源性微粒^[37]、合体滋养细胞源性微粒^[38]上检测到 TFPI。某些疾病如急性心肌梗死^[39]、糖尿病^[40]的患者的循环血中亦检测到 TFPI。这些印证了人们的假设。MP 在血液中所具有的促凝/抗凝活性, 则可能有 TF/TFPI 的比值所决定。一项临床研究表明, 正常健康对照者这一比值的中位数在 0.4, 而肿瘤患者的这一比值的中位数则升高到 0.9^[41], 通过对这一比值的测量, 可以评估患者血栓发生的风险。EMPs 的抗凝活性还通过调节蛋白 C 系统来实现。EMPs 表面表达的 TM、EPCR, 分别为凝血酶和蛋白 C 的受体, TM 与凝血酶结合后可降低凝血酶活性, 并加强其激活蛋白 C 的活性。激活的蛋白 C 与 EPCR 阳性的 MP 结合, 灭活 FV a、FVIIIa, 从而下调凝血酶的生成^[42]。

4 MP 样本的准备及检测

目前对于 MP 的样本准备及检测一直缺乏一个统一的标准。抽血、离心、转运、储存、抗凝剂的使用等各个方面都可能影响到实验的结果。抽血针头的大小为 19 号或 21 号, 针头过小, 会导致剪切力增加, 有可能导致红细胞源性 MP 的产生^[43]。在抗凝剂的选择上, 3.2% 或者 3.8% 的柠檬酸钠是最常使用的抗凝剂。有研究发现, 使用肝素抗凝比使用柠檬酸钠抗凝, 所检测到的总 MP 数量明显升高^[44]。也有人发现, 在 60min 时间, 膜联蛋白 V 阳性的 MP 和 PS 的浓度在柠檬酸钠抗凝管比 EDTA 抗凝管要高, 所以建议如果标本在采集后不能立即离心, 那么采用 EDTA 抗凝更好^[45]。对于离心的速度, 学者们也有不同看法。但总的来说, 有两步十分关键: (1) 低速离心 (200-13000g) 去除细胞、血小板并使 MP 富集在上清中; (2) 高速离心 (18000~100000g) 使 MP 沉淀^[46]。既往的学者们采用的实验方案也不尽相同。因而, 实验方案的标准化显得特别重要。

2013 年国际血栓与止血协会工作组 (ISTH/SSC) 推荐了一项标准化的试验方案, 其主要步骤包括^[47]: (1) 早晨 8~11 点空腹采血; (2) 使用 21 号针头, 于肘静脉处采用, 止血带不可过紧。最初的 2~3ml 血液

不用于 MP 的检测。(3) 使用容量至少为 3.5ml 的含 3.2% 柠檬酸钠的塑料采血管收集血液；(4) 有可能的话直接在实验室采血，如果必须转运标本，则应把标本垂直置于盒内固定并平稳转运，温度为室温(20~25°C)；(5) 样品 2500g 室温下离心 15min，收集上层血浆，并留至少 1cm 血浆层以防混入血小板；(6) 收集的血浆再次在室温下 2500g 离心 15min，收集上清液，得到无血小板血浆 (PFP)。(7) 如不能在 2h 内分析使用，则将血浆于 -80°C 冷冻保存。

5 糖尿病与微粒

5.1 糖尿病的高凝状态 在糖尿病患者中，心血管疾病仍然是其死亡原因的首位。75%~80% 的糖尿病患者最终死于心血管并发症，糖尿病患者的动脉粥样硬化血栓形成较非糖尿病患者增加^[48]。目前认为其原因主要包括：内皮细胞功能紊乱、血小板激活、以及糖尿病患者的高凝状态。糖尿病患者内皮功能紊乱的原因主要包括：胰岛素抵抗及高血糖使糖尿病患者氧化应激增加。从而导致晚期糖基化产物 (advanced glycation end products, AGEs)、非对称性二甲基精氨酸 (asymmetric dimethylarginine, ADMA) 生成增加，脂质过氧化增加。AGEs 与血管内皮细胞上的相应受体结合，导致内皮细胞表达 ICAM-1、VCAM-1、TF、促炎症因子 IL-1 及 TNF 均表达增加^[49]。而 ADMA 是血管内皮细胞 NO 合酶 (eNOS) 抑制剂，ADMA 增加使血管内皮 NO 生成减少，血管舒张功能紊乱。另外，循环中的内皮祖细胞对于维持血管稳态、血管代偿有非常重要的作用，研究人员发现 2 型糖尿病患者循环内皮祖细胞数量及功能均较正常对照组低^[50]。糖尿病患者高凝状态的机制亦十分复杂，目前认为主要包括：胰岛素抵抗促时纤溶酶原激活物抑制物-1 (plasminogen activator inhibitor-1, PAI-1) 增加，抑制糖尿病患者纤溶活性；高血糖时患者呈低度炎症状态，其循环中 IL-6 增加，刺激肝脏生成纤维蛋白原增加；另外人们发现糖尿病患者血液中多种凝血相关因子的增加，包括：TF、FVII 促凝活性 (FVII:c)、凝血酶^[51]。

5.2 糖尿病患者微粒与其高凝状态 由于微粒具有的如上所述的促凝活性，研究者们相信微粒在糖尿病的高凝机制中也发挥了很大的作用。许多研究表明，糖尿病患者血液中微粒总量升高^[52]，EMPs 升高^[53]，MP 的促凝活性增加。与健康对照相比，2 型糖尿病患者血浆 PMPs 明显升高，且不与是否合并肥胖相关^[54]。1 型糖尿病患者，PMPs 亦明显升高，且与超敏 CRP、糖化血红蛋白、颈动脉内膜中层厚度 (carotid intima media thickness, CIMT) 呈明显正相关，研究认为 PMPs 升高可作为预测 1 型糖尿病患者微血管并发症及早期动脉硬化的标记物^[55]。合并不同慢性并发症的糖尿病患者，其血浆微粒亦有不同特点。合并糖尿病足的 2 型糖尿病患者循环 MP、PMP 浓度较正常对照组增加，合并冠心病、糖尿病足、严重糖尿病足的 T2DM 患者血浆微粒表达的 TF/TFPI 比值较健康对照组明显升高，该比值越高，微粒的促凝活性越强。在与严重糖尿病足患者的 MP 共培养后，HUVEC 细胞 TF-mRNA 表达量、TF 生成增加达 5 倍，与糖尿病视网膜病变患者、合并心脏病患者的 MP 共培养后，其增加达 3 倍^[56]，这些结果说明糖尿病患者的 MP，特别是合并严重糖尿病足的患者的 MP，具有很高的促凝活性。但 MP 的促凝活性实验结果不尽相同。Diamant 等^[57] 使用去纤维蛋白、去 MP 的正常人的血浆，与 2 型糖尿病患者、健康对照者的 MP 混合，检测凝血酶生成的量，发现糖尿病组生产的量更少。所以，糖尿病患者的微粒在凝血过程中发挥的具体作用有待我们进一步研究。

6 展望

近年来，对微粒的研究不断深入，但其释放的具体机制尚不完全明了，对其检测的标准化尚存在不同看法 (如，对于贫血小板血浆 (platelet poor plasma, PPP)、无血小板血浆 (platelet free plasma, PFP) 的制备和定义)，各实验室结果也存在较大差异。也有研究学者希望能根据微粒的促凝活性，从治疗方面入手，减少某种微粒的生成、减低其抗凝活性，以达到减弱疾病高凝状态的目的。但由于其生成释放原理不完全明了，从患者血液中直接分离微粒有一定困难，目前研究成果不多^[58]。未来，在研究某些疾病过程中微粒的上具体分子的作用机制、治疗靶点等方面可能会有更多成果。

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