激动剂诱导血小板活化机制及抗血小板药物研究进展

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摘 要 血小板在凝血酶、胶原、血小板活化因子、花生四烯酸、二磷酸腺苷、肾上腺素及5-羟色胺等激动剂作用下产生一系列复杂的信号转导,可引起血小板包括形变、颗粒内容物释放、聚集等生理反应。血小板在血管损伤部位的活化聚集是正常凝血的关键步骤之一,但过度的血小板聚集会形成血栓,从而引发急性缺血事件。因此,抗血小板治疗是血栓性疾病治疗的重要方向之一。目前的抗血小板药物主要针对活化通路中的受体或者信号分子,通过抑制受体或者阻断信号传播而发挥作用。本文对常见诱导剂诱导血小板活化的靶点和作用机制进行介绍,并对受体拮抗药和其他抗血小板药物进行综述,为进一步研究抗血小板药物提供参考。

关键词 血小板活化;激动剂;受体;抗血小板药物;凝血酶;胶原;血小板活化因子;花生四烯酸;二磷酸腺苷

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Mechanism of agonist-induced platelet activation and research progress of anti-platelet drugs

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Abstract Thrombin, collagen, platelet activating factor, arachidonic acid, adenosine diphosphate, adrenaline, serotonin and other platelet agonists can stimulate platelet to generate a series of complex signal transduction and then cause platelet physiological responses including deformation, granule release and aggregation etc. Although platelet activation and aggregation at sites of vascular injury is an essential step in coagulation process, excessive platelet accumulation leads to the formation of occlusive thrombi, which are responsible for acute ischemic events. Therefore, anti-platelet therapy is an important approach for the treatment of thrombotic diseases. Currently used anti-platelet drugs, which primarily act through receptors and/or signaling molecules in activation pathways, achieving their effects by inhibiting platelet receptors or blocking signal transmission. In this review, the targets of the above-mentioned platelet agonists and their specific activation mechanisms are introduced; receptor antagonists and other anti-platelet drugs are also summarized.

Key words platelet activation; agonists; receptors; anti-platelet drugs; thrombin; collagen; platelet activating factor; arachidonic acid; adenosine diphosphate

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血小板是一种有质膜无细胞核,形状无规则的血细胞,是血液中的一种有形成分,主要作用是保持血管完整性以及防止血管损伤后出血。正常的血小板活化聚集是一种必要的防御机制,但过度聚集导致的血栓形成会阻碍血流,严重的甚至引起急性心肌梗死、卒中或者其他一些血栓性疾病[1]。因此,抗血小板治疗是血栓性疾病治疗的重要方向之一[2]。

血小板的活化与激动剂密切相关。血小板激动剂包括凝血酶(thrombin,THR)、胶原、血小板活化因子(platelet activating factor,PAF)、花生四烯酸

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(arachidonic acid, AA)(代谢产物)、二磷酸腺苷(adenosine-5′-diphosphate, ADP)、肾上腺素及 5-羟色胺(5-hydroxytryptamine, 5-HT)等在血小板表面都有相应的受体。激动剂通过与特定的受体结合,引发一系列复杂的信号转导,导致血小板形变、黏附、聚集而形成血栓。抗血小板药是抗血栓药的重要组成部分,其主要通过阻断与血栓形成相关的膜受体或干扰细胞内信号通路两种途径来实现[3],新型抗血小板药物的发现和发展是一个热门领域。本文首先对血小板常见活化诱导剂激活血小板的机制进行介绍,然后从相关受体拮抗剂出发,总结抗血小板药的研究进展,为抗血小板药的后续研究及临床用药提供参考。

1　血小板常见诱导剂介导的血小板活化通路

血小板内的信号传递起始于诱导剂诱导血小板表面各种受体的激活。不同诱导剂引起血小板聚集的能力不同:强诱导剂凝血酶、胶原可引起不依赖于颗粒内容物的聚集反应;中等强度的诱导剂 PAF、AA 对血小板聚集的诱导能力稍弱于前者;弱诱导剂 ADP、5-HT 若要引起不可逆的聚集反应需要颗粒内容物分泌后的协助作用;而肾上腺素只能在超过生理浓度下发挥作用[4]。

1.1　凝血酶

凝血酶(THR)是一种丝氨酸蛋白酶,除了促进凝血因子Ⅴ,Ⅷ,Ⅺ和Ⅻ产生以及裂解纤维蛋白原转化为纤维蛋白外,还参与血管张力调节、平滑肌细胞增殖和炎症反应[5],同时也是血小板活化最有力的激动剂之一。THR 活化血小板时涉及两种细胞膜成分:G 蛋白耦合的 7 次跨膜受体(protease activated receptors, PARs)和富亮氨酸重复家族(leucine-rich repeat, LRR)受体(GPⅠb/Ⅸ/Ⅴ复合物)。两者通过不同的途径活化血小板,GPⅠb/Ⅸ/Ⅴ复合物主要参与血小板黏附,PARs 则参与聚集。

PARs 是一类蛋白酶激活受体,这些受体通过一种独特机制刺激血小板,将一个胞外蛋白水解事件转换为跨膜信号:THR 结合 PAR 的 N-末端胞外区,切割并暴露一个新的 N-末端,通过折叠,成为不依赖 THR 的 PAR 配体。PARs 存在 4 个独立的 PAR 受体,其中 PAR-1, PAR-3, PAR-4 均为对 THR 敏感的受体,PAR-2 则是胰蛋白酶及凝血因子Ⅶa 和 Xa 敏感受体,同时 PAR-2 与 PAR-3 都不在人类血小板上表达[6]。PAR-1 和 PAR-4 调节 THR 对血小板的作用中存在一个双受体系统:PAR-1 介导血小板对 THR 的初始应答,激活的 PAR-1 信号迅速脱敏,而 PAR-4 信号则比较持续,但 PAR-1 相对来说更加重要,因为 PAR-1 能在低浓度 THR 的情况下激活血小板,而 PAR-4 只在高浓度 THR 条件下才能发挥作用[7]。

PAR-1 耦合于 $G_{12/13}$、Gq 蛋白,是凝血级联反应的主要效应蛋白。$G_{12/13}$的 α 亚基结合到 Rho 鸟嘌呤核苷酸交换因子(Rho guanine nucleotide exchange factors, RhoGEFs),引起可能参与血小板形状改变的细胞骨架反应;Gq 和 Gi 通路使细胞内 Ca^{2+}浓度升高,环磷酸腺苷(cAMP)水平降低。PAR-4 激活 Gq,也可能激活 $G_{12/13}$信号通路。虽然 PAR-1 和 PAR-4 不耦合于 Gi,但可以引发致密颗粒中的 ADP 释放,激活 Gi 通路的 P_2Y_{12}受体,通过信号转导,最终增强 PAR-1 介导的血小板不可逆聚集[9-10](图 1)。

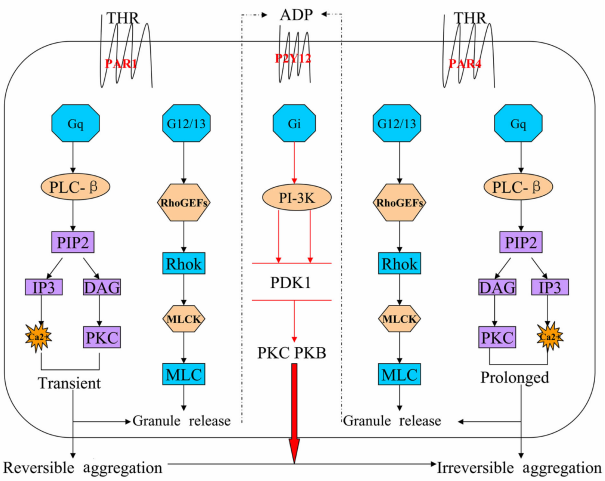

Figure 1　PARs and platelet activation in human[8]

1.2　胶　原

丰富的内皮下基质胶原蛋白是一个重要的生理血小板激动剂,在血管损伤后,暴露的胶原纤维有助于血栓的初步形成。血小板表面有两个胶原受体:整合素 α2β1 和血小板膜糖蛋白Ⅵ(glycoprotein Ⅵ, GP Ⅵ)。胶原诱导的血小板活化是通过激活低亲和力免疫受体糖蛋白 GP Ⅵ集群以及第 2 胶原受体 α2β1 活化后的后续内外信号调节互相配合协作的过程(图 2)。

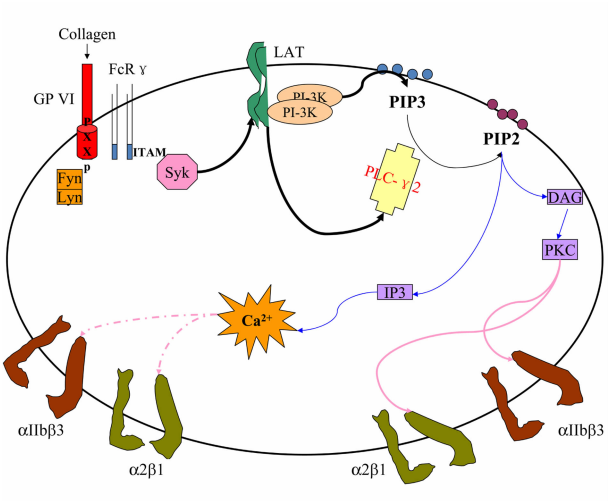

Figure 2 Platelet signaling through collagen[11]

GP Ⅵ是免疫球蛋白(immunoglobulin superfamily,Ig)超家族成员,是血小板特异性、低亲和力、高效力的胶原受体[12]。GP Ⅵ没有固有的信号传导能力,必须联合 IgG Fc 受体(FcR)才能传导信号。FcR 的γ链是共价连接的同型二聚体,包含一种受体信号转导亚基——免疫受体酪氨酸活化基序(immunoreceptor tyrosine-based activation motifs,ITAM)。GP Ⅵ复合物与 FcRγ 链通过蛋白跨膜结构域中的精氨酸和天冬氨酸盐桥连接,一经连接,酪氨酸激酶介导的信号转导途径便被激活,受体聚集成簇,ITAM 被 Src 家族(Lyn 和 Fyn)酪氨酸激酶磷酸化,其产物聚集到质膜上,引起跨膜接头蛋白(linker for activation of T cells,LAT)酪氨酸磷酸化,组装信号蛋白复合物,刺激磷脂酶 C(phospholipase C,PLC)磷酸化激活[13]。PLC-γ_2激活后产生第 2 信使三磷酸肌醇酯(inositol-3-phosphate,IP3)和甘油二酯(diacylglycerol,DAG),DAG 可以激活蛋白激酶 C(protein kinase C,PKC),调节丝氨酸/苏氨酸(Ser/Thr)磷酸化,使血小板活化,调整整合素 αIIbβ3 形态,结合纤维蛋白原。

α2β1 是除 αIIbβ3 外血小板上第 2 重要的整合素,在血小板上也叫血小板膜糖蛋白Ⅰa/Ⅱa(glycoprotein,GPIa/IIa),能调节稳定的胶原黏附,是血小板上的主要胶原受体。虽然已经被认定是胶原诱导血小板的受体,但有关 α2β1 受体信号的作用仍然具有很多争议[14]。最近的研究表明,α2β1 的活化机制与其他整合素相似:通过一些相互反应使其最终脱离相应的跨膜结构域而变成活性受体[15]。胶原蛋白结合整合素 α2β1 不仅有助于稳定附着力加强 GP Ⅵ结合,还能产生目前不确定生理意义的弱细胞内信号同时引发 GP Ⅵ诱导的"外-内"信号转导[16]。

1.3 血小板活化因子

血小板活化因子(PAF)是由白细胞、血小板、内皮细胞及单核巨噬细胞等在特异性刺激下释放的一种内源性强效生物活性磷脂,参与细胞内很多信号传导,发挥着多种多样的生理作用。

PAF 发挥生物学效应主要是通过与细胞膜表面的 PAF 受体(PAFR)结合来实现。PAFR 是 G 蛋白耦联受体家族的成员,虽然在某些功效上与 Gi 机制——抑制蛋白激酶 A(protein kinase A,PKA)活性,阻止 ATP 向 cAMP 转换等相关联[17],但目前认为 PAFR 主要耦合于 Gq 蛋白:当 PAF 和受体结合后,通过 Gq 蛋白激活 PLC,分解磷脂酰肌醇 4,5 二磷酸(phosphatidylinositol- 4,5-bisphosphate,PIP2)产生 IP3 和 DAG,前者引发 Ca^{2+} 动员,后者激活 PKC,引发丝裂原活化蛋白(mitogen-activated protein,MAP)激酶以及 TXA_2 通路磷脂酶 A_2(phospholipase A_2,PLA_2)活化,最终导致血小板形变和聚集,血管收缩,微循环障碍以及炎性反应[18-19](图 3)。

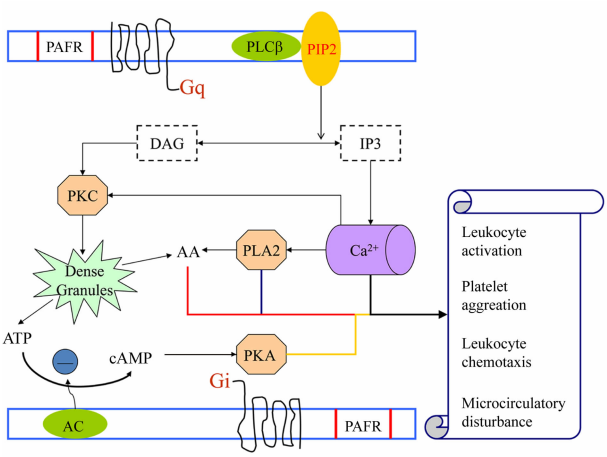

Figure 3 PAF-mediated platelet signaling pathways

1.4 花生四烯酸

花生四烯酸(AA)存在于血小板及人体绝大多数细胞,一般以磷脂形式酯化在细胞膜中,不游离存在。血小板激活后,胞内 Ca^{2+} 水平升高,刺激 PLA_2 活化,在 PLA_2 作用下,AA 从膜磷脂中游离出来。血小板膜上并无 AA 受体,外源性的 AA 作用于血小板时经由环氧合酶(cyclooxygenase,

COX)作用转化为前列腺素 G_2(prostaglandin G_2,PGG_2)和 PGH_2,在 COX-1 或者血栓素合成酶(thromboxane synthase,TX)作用下合成血栓素 A_2(thromboxane A_2,TXA_2),在血管内皮细胞中由前列腺素内过氧化物合酶-2(prostaglandin H synthase 2,PGHS-2 也称为 COX-2)合成 PGI_2[20-21]。

PGI_2 结合到血小板膜 Gαs 耦合的 IP 受体刺激膜生成腺苷环化酶(adenylate cyclase,AC),增加 cAMP 水平,激活 cAMP 依赖性 PKA;TXA_2 与血小板膜上的 TXA_2 受体(TP)结合,以自分泌和旁分泌方式产生血小板活化的反馈回路放大。PGI_2 和 TXA_2 分别激活和抑制 AC,二者的动态平衡可以控制正常的止血功能[22]。

血小板上存在两种 TXA_2 的剪接变异受体:TPα 和 TPβ。这两种受体都能被 PGG_2、PGH_2 激活。TPα 耦合于 Gq 和 $G_{12/13}$蛋白。Gq 使 PLCβ 活化,分解 PIP2 为 IP3 和 DAG,使 Ca^{2+} 浓度升高;$G_{12/13}$蛋白也能使细胞内 Ca^{2+} 浓度升高,但 G_{12}不能使 PLCβ 活化,其内钙升高与 IP3 无关[23],$G_{12/13}$的 α 亚基结合到 RhoGEFs,提供了可能参与血小板形状改变的细胞骨架反应;G_{13}则与前列环素所致 TP 受体的磷酸化有关[24]。TPβ 与 Gi 蛋白耦合,能激活 PI3K 引起 GPⅡb/Ⅲa 活化,导致血小板聚集。图 4 为 AA 合成、转化以及对血小板作用过程简易流程图。

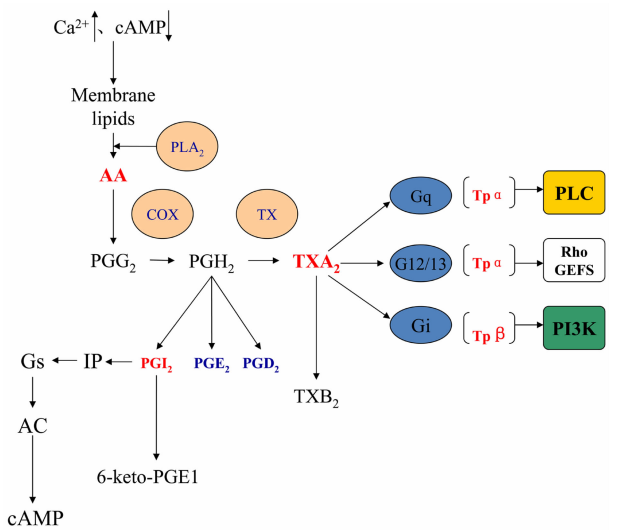

Figure 4　AA-induced platelet aggregation pathway and related factors

1.5　二磷酸腺苷

二磷酸腺苷(ADP)是第 1 个已知的低分子血小板弱聚集诱导剂。ADP 以较高的浓度储存在血小板致密颗粒中,当血小板活化后,ADP 从致密颗粒中释放,作为二次激动剂,通过作用于多种受体,增强自身及其他诱导剂对血小板的诱导作用并有助于血栓的稳定化(图 5)。

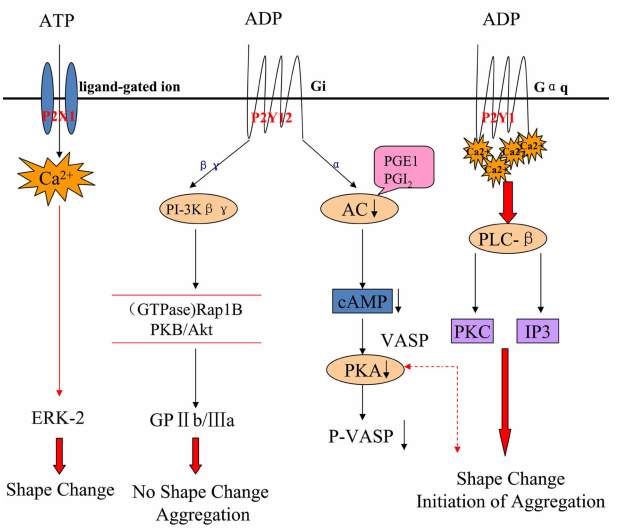

Figure 5　ADP and ATP signaling pathway in platelet activation and aggregation[8]

ADP 激活血小板主要依靠两个 G 蛋白耦合受体:与 Gq 蛋白耦合的 P_2Y_1 和与 Gi 尤其是 Gi_2 耦合的 P_2Y_{12}。事实上,有研究提出血小板中存在 3 个不同的 P2 受体亚型,除开前两个,还有配体门控离子通道受体 P_2X_1,P_2X_1 很长一段时间被认为是 ADP 受体,但后来证实 P_2X_1 只被 ATP 激活[25]。激活 P_2Y_1 受体会加强细胞内 Ca^{2+} 动员,促进 Ca^{2+} 释放,引起血小板形变以及比较短暂的血小板聚集。虽然引起聚集的作用比较弱,但 P_2Y_1 却是 ADP 和胶原诱导血小板活化起始步骤的关键受体之一[26];P_2Y_{12}是最重要的 ADP 受体,激活 P_2Y_{12}受体能抑制 AC 活性,降低 cAMP 浓度,使 PKA 活性降低,PKA 活性降低后引起靶蛋白例如血管刺激扩张磷蛋白(vasodilator stimulated phosphoprotein,VASP)等磷酸化减少,解除 VASP 对细胞变形和聚集的抑制作用[27],增强血小板聚集。虽然 P_2X_1 的激活在 ADP 诱导的血小板聚集中基本不起作用[28],但是激活后的 P_2X_1 能使 Ca^{2+} 内流并诱导细胞外信号调节激酶(extracellular signal-regulated kinase,ERK-2)活化,这对小动脉中剪切力条件下的胶原诱导的血栓形成很重要[29]。

1.6 其他弱诱导剂

5-羟色胺(5-HT)除了作为神经递质外,也能参与血小板聚集以及平滑肌收缩等过程,在动脉血栓形成过程中起关键作用[30]。5-HT通过胃肠道的肠嗜铬细胞合成后进入血液,迅速被血小板吸收,储存于致密颗粒内部。静息状态下,外周血液中的5-HT含量很少,血小板在血管损伤部分活化后,血小板致密颗粒会释放5-HT。5-HT受体有不同的受体亚型,已发现14种5-HT受体亚型[31],血小板内主要的血清素受体是$5\text{-}HT_{2A}$,属于G蛋白耦联受体家族,它与5-HT结合之后,引起Ca^{2+}信号改变,血小板活化信号放大,导致血小板形状的变化和弱的血小板可逆聚集。同时,5-HT还能增强其他诱导剂,例如胶原、ADP及肾上腺素等对血小板的激活作用[32]。

肾上腺素不能单独激活血小板,只能通过肾上腺素能受体α2A来增强其他诱导剂的效果。肾上腺素能抑制cAMP形成,刺激TXA_2水解磷酸肌醇,对PLC没有影响,也不会引起血小板形状变化。研究表明肾上腺素能受体α2A优先耦合到G蛋白耦联受体的Gi家族(尤其是Gz)[33]。

2 抗血小板药物

抗血小板治疗是血栓性疾病治疗的主要方向,很多抗血小板药物如阿司匹林、氯吡格雷等都是目前动脉粥样硬化等急性心脑血管疾病的主要治疗药物。本文从初级受体拮抗剂(ADP受体,THR受体,AA受体,PAF受体,5-HT受体)、纤维蛋白原受体拮抗剂(膜糖蛋白ⅡB/Ⅲa受体)、磷脂酶抑制剂(PDE)以及其他新靶点药物几个方面将目前临床应用广泛的抗血小板药和正在进行研究的一些其他抗血小板靶点药物进行归纳总结。

2.1 ADP受体阻滞剂

ADP的3个受体在血小板上表达数量大小依次为:P_2Y_{12}、P_2Y_1、P_2X_1。从作用程度来说,P_2Y_{12}是ADP诱导血小板聚集反应中最重要的受体[34],所以目前的ADP受体抑制剂主要是针对P_2Y_{12}。

噻氯吡啶(ticlopidine)、氯吡格雷(clopidogrel)和普拉格雷(prasugrel)是噻吩并吡啶类抗血小板药物,都是P_2Y_{12}受体抑制剂,而且3种药物都必须先经过生物肝细胞色素酶P450系统代谢成为有活性的代谢产物(目前尚未分离获得活性代谢产物)才能发挥作用。这类药物能与P_2Y_{12}受体外半胱氨酸残基共价结合形成二硫键,所以是不可逆抑制剂。

由于噻吩并吡啶类药物都是前药,在治疗的时候会有迟滞性,所以在此类药物之后,能够应用于急性心肌梗死突发状况的药物——竞争性受体拮抗剂,成为ADP受体抑制剂的研究重点。ATP及其三磷酸类似物是P_2Y_{12}受体拮抗剂,基于此,ATP核苷酸类似物抗血小板药(AR-C复合物)成为一种新的竞争性P_2Y_{12}受体拮抗剂。AR-C复合物主要包括AR-C66096和AR-C69931MX,AR-C69931MX(坎格雷洛,cangrelor)在人体和动物中的研究广泛[35]。2011年批准上市的替格瑞洛(ticagrelor)是将坎格雷洛的一个嘌呤核以及核糖部分做进一步的修饰,消除磷酸基团后得到的新药,在降低急性冠脉综合症患者心血管死亡和心脏发作方面效果优于氯吡格雷[36]。

BX667是一种新开发的具有P_2Y_{12}受体可逆性抑制作用的化合物,在动物实验中与氯吡格雷效果相当而且能明显降低出血概率,在体内通常会被酯酶水解为相应的酸BX048,研究表明两种物质均有P_2Y_{12}拮抗作用[37],但是近年来却未见该实验的进一步报道。三环苯并噻唑类药物依诺格雷(elinogrel),CT50547等也能竞争性拮抗P_2Y_{12}受体[38]。INS复合物属于腺嘌呤核苷酸类似物,目前正处于研究阶段,作为具有良好耐受性、选择性的可逆P_2Y_{12}受体抑制剂,是抗血小板药的新方向[39]。

P_2Y_1受体抑制剂基本上都是核苷酸类似物。MRS复合物的开发源于天然的二磷酸核苷酸,例如腺苷-3′-5′-二磷酸(A3P5P)等选择性P_2Y_1受体抑制剂,MRS2179是最早的代表药物[40]。后来合成的二磷酸受体拮抗剂MRS2279,MRS2500采用了刚性的结构替代以前的核糖环,使药物对受体的亲和力以及对核苷酸酶的结合力都大大增强,成为高亲和力的选择性P_2Y_1受体拮抗剂。

ATP门控受体P_2X_1在高剪切应力下的THR诱导的血小板聚集过程中起着重要的作用[41]。α,β-亚甲基ATP(αβMeATP)是P_2X_1选择性激动剂,苏拉明(suramin)是高亲和力P_2X_1拮抗剂的先导化合物,一系列二价和四价苏拉明类似物对αβMeATP诱导的血小板Ca^{2+}动员以及形变的实验探索结果显示这些类似物(NF846,NF449)能够选择性抑制

P_2X_1 受体,其中作用最好的是 NF846[42]。ADP 通路相关受体拮抗剂总结如表 1。鉴于药物研发领域发展迅速,本文所列除已上市药物外,其他处于研究阶段的药物的研究进展仅供参考。

2.2　THR 相关抑制剂

有研究表明,缺乏主要凝血酶受体的小鼠不会产生自发性出血,但是缺乏黏附受体(GPⅡb/Ⅲa GPⅠb/Ⅸ)的小鼠就会出现出血现象[56]。由此推测,凝血酶在止血过程中只是选择性作用于血栓形成[57]。如果此假设成立,那么针对凝血酶通路的抗血栓药物在治疗疾病的过程中就可避免引发出血过多事件。目前靶向凝血酶通路的药物(如表 2)主要作用于 3 个位点:凝血酶 PAR-1 受体,凝血酶以及凝血因子Ⅹa。

Table 1　Properties of ADP receptor relative blockers

Agent	Class	Formula	Mechanism	Administration	Development	Reference
Ticlopidine	Thienopyridine	$C_{14}H_{14}ClNS$	P_2Y_{12} inhibitor Pro-drug irreversible	*po*	Approved(Ticlid®)	[43]
Clopidogrel	Thienopyridine	$C_{16}H_{16}ClNO_2S$	P_2Y_{12} inhibitor Pro-drug irreversible	*po*	Approved(Plavix®)	[44]
Prasugrel	Thienopyridine	$C_{20}H_{20}FNO_3S$	P_2Y_{12} inhibitor Pro-drug irreversible	*po*	Approved(Effient®)	[45]
Cangrelor	ATP analog	$C_{17}H_{25}C_{12}F_3N_5O_{12}P_3S_2$	P_2Y_{12} antagonist direct-acting, competitivereversible	iv	Phase III AR-C69931 MX	[46]
Ticagrelor	Cyclopentyl triazolopyrimidine	$C_{23}H_{28}F_2N_6O_4S$	P_2Y_{12} antagonist direct-acting, competitivereversible	*po*	Approved(Brilinta®)	[47]
AR-C66096MX	ATP analog	$C_{14}H_{22}F_2N_5O_{12}P_3S$	P_2Y_{12} antagonist direct-acting, competitivereversible	iv	Phase II	[48]
Elinogrel	Tricyclic benzothiazole	$C_{20}H_{15}ClFN_5O_5S_2$	P_2Y_{12} antagonist direct-acting, competitivereversible	*po*,iv	Phase III (PRT060128)	[49]
BX667		$C_{29}H_{38}N_4O_8$	P_2Y_{12} antagonist direct-acting reversible	*po*	Preclinical (No progress)	[37]
CT50547	Tricyclic benzothiazole derivative	$C_{22}H_{20}N_4O_8S_4$	P_2Y_{12} antagonist direct-acting reversible	*po*	Preclinical	[50]
INS-50589	Adenosine-monophosphate analog	$C_{22}H_{25}N_6O_8P$	P_2Y_{12} antagonist direct-acting, competitivereversible	iv	Phase II	[39]
MRS2179	Nucleotide analog	$C_{11}H_{17}N_5O_9P_2$	P_2Y_1 antagonist direct-acting	iv	Research tool	[40]
MRS2500	Nucleotide analog	$C_{13}H_{18}N_5O_8P_2I$	P_2Y_1 antagonist direct-acting,high affinity	iv	Research tool	[51]
MRS2279	Nucleotide analog	$C_{13}H_{18}ClN_5O_8P_2$	P_2Y_1 antagonist direct-acting,high affinity	iv	Research tool	[52]
MRS2496	Acyclic nucleotide	$C_{10}H_{14}ClN_5O_6P_2$	P_2Y_1 antagonist	iv	Research tool	[53]
A2P5P	Diphosphate nucleotide	$C_{10}H_{15}N_5O_{10}P_3$	P_2Y_1 inhibitor direct-acting, competitiveversible	iv	No progress	[54]
A3P5P	Diphosphate nucleotide	$C_{10}H_{15}N_5O_{10}P_3$	P_2Y_1 inhibitor direct-acting, competitiveversible	iv	No progress	[54]
NF449	Suramin analogs	$C_{41}H_{24}N_6Na_8O_{29}S_8$	P_2X_1 inhibitor direct-acting	*po*	Research tool	[55]
NF864	Suramin analogs	$C_{57}H_{28}N_6Na_{12}O_{42}S_{12}$	P_2X_1 inhibitor direct-acting	*po*	Research tool	[42]

典型的 PAR-1 受体抑制剂有两种:Vorapaxar 和 Atopaxar。Vorapaxar(2014 年 5 月获 FDA 批准上市)是一种源于天然产物喜巴辛的 PAR-1 受体拮抗剂,对受体具有高亲和力。分子动力学模拟显示,Vorapaxar 能使受体一直处于无活性的构象[58]。与 Vorapaxar 相比,Atopaxar 是一种具有更短半衰期、能更早被胃肠代谢的小分子合成药物,Atopaxar 可以在非常接近栓系结合位点的地方抑制凝血酶受体激活肽(thrombin receptora activator peptide,TRAP)[59]。除了直接阻断血小板 PAR-1 受体外,Atopaxar 还能引起一系列血小板表面主要受体,包括 GPIIb/IIIa,血小板内皮细胞黏附分子(platelet endothelial cell adhesion molecule,PECAM-1),起到强烈的抗血小板效果[60]。

希美加群(ximelagatran)是直接凝血酶抑制剂中第一个可以口服的前药,在肝脏和其他很多组织中通过脱烷基和脱羟基化被转换为美拉加群后才发挥作用[61]。2004 年希美加群在很多国家已经上市,但 2006 年 EXTEND 临床研究显示,接受治疗的患者中出现了严重的肝损伤,阿斯利康公司对其进行紧急召回,目前该药已不再使用[62]。达比加群(dabigitran)是继希美加群后一种新的口服抗凝血剂,通过直接抑制凝血酶起作用,是华法林后的新一代抗凝剂[63],其疗效和安全性,获得全球最

大型心房颤动转归临床试验——RE-LY 研究的数据支持,被誉为近 60 年来防卒中的最佳药物。我国在 2013 年 3 月对其颁发进口药品注册证。

间接凝血酶抑制剂肝素通过与抗凝血酶络合使凝血辅助因子由慢到快地转变为凝血酶抑制因子,起到增强抗凝酶Ⅲ活性,激活肝素辅助因子Ⅱ,并且促进纤溶系统激活,抗血小板聚集等作用[64]。

中断凝血因子Xa 后,凝血级联反应的内外两条途径都将被中断,凝血酶形成以及血栓发展将被抑制。Xa 抑制剂分为两种,磺达肝癸钠(fondaparinux)是间接的,对Xa 起抑制作用需要抗凝血酶Ⅲ的辅助作用;利伐沙班(rivoraxaban)是由拜耳研发和生产的第 1 个高度选择性直接凝血因子Xa 抑制剂,口服生物利用度高,起效迅速,对自由存在的Xa 和与凝血酶原酶复合物结合的Xa 有同等的抑制效力[65],FDA 于 2011 年 11 月批准该药上市,用于预防脑卒中患者的非瓣膜性房颤。依度沙班(edoxaban)也是直接凝血因子Xa 抑制剂,能够可逆性抑制凝血因子Xa,于 2011 年 4 月在日本批准用于预防骨科大手术后静脉血栓栓塞(VTE),同年 7 月在日本以商品名 Lixiana 上市。但是在其他地方,包括欧洲和美国,依度沙班目前正处于Ⅲ期临床,尚未获批用于任何适应证[66]。阿哌沙班(apixaban)是另外一个直接凝血因子Xa 抑制剂[67],是一种口服的选择性活化Xa 因子抑制剂,由辉瑞与百时美施贵宝联合开发。Apixaban 能预防血栓,但出血的不良反应低于老药华法林,用于接受过髋部或膝部置换手术患者的血栓预防。2012 年 12 月 FDA 批准该药在美国上市。

Table 2 Properties of thrombin relative blockers

Agent	Class	Formular	Mechanism	Administration	Development	Reference
Vorapaxar	Tricyclic himbacine derivative	$C_{29}H_{33}FN_2O_4$	PAR-1 antagonist high affinity, competitive reversible	*po*	Approved(Zontivity®)	[68]
Atopaxar	Bicyclic amidine	$C_{28}H_{36}FN_3O_5$	PAR-1 antagonist competitive, reversible	*po*	Phase II(E5555)	[69]
Ximelagatran	Amidino compound	$C_{24}H_{35}N_5O_5$	Direct thrombin inhibitor Pro-drug	*po*	Draw back(Exanta®)	[61]
Dabigitran	Bicyclic amidine	$C_{34}H_{41}N_7O_5$	Direct thrombin inhibitor	*po*	Approved(Pradaxa®)	[70]
Heparin	Sulfated glycosaminoglycan	$C_{12}H_{19}NO_{20}S_3$	Indirect thrombin inhibitor	iv	Approved	[71]
Fondaparinux	Low molecular weight heparin analogs	$C_{31}H_{43}N_3Na_{10}O_{49}S_8$	Indirect Xa inhibitor	iv	Approved(Arixtra®)	[72]
Rivoraxaban	Oxazolidinone derivatives	$C_{19}H_{18}ClN_3O_5S$	Direct Xa inhibitor	*po*	Approved(Xarelto®)	[65]
Apixaban	Pyrazolopyridines formamide	$C_{25}H_{25}N_5O_4$	Direct Xa inhibitor	*po*	Approved(Eliquis®)	[73]
Edoxaban	p-Toluenesulfonate	$C_{24}H_{30}ClN_7O_4S$	Direct Xa inhibitor	*po*	Approved(Lixiana®)	[74]
Warfarin	Coumarin	$C_{19}H_{16}O_4$	Inhibition of vitamin K coagulation factors	*po*	Approved(Coumadin®)	[75]

2.3 AA 通路相关抑制剂

阿司匹林(aspirin)无疑是目前应用最广的血栓栓塞性疾病预防药,其通过选择性乙酰化 COX-1 丝氨酸残基 529 位点(Ser529),抑制 COX-1 的活性,影响 AA 生成 TXA_2,抑制血小板聚集。大量的临床试验和数据分析都证实阿司匹林可以很好地预防动脉粥样硬化患者的二次缺血复发事件[76]。但是,阿司匹林的用药剂量一直具有争议,且副作用和抵抗效应较多[77]。

为了改善阿司匹林的效果,从 NO 供体药物出发,研制了阿司匹林衍生物。NCX4016,2-(乙酰氧基)苯甲酸 3-(硝基氧乙基)苯基酯,由乙酰水杨酸与 NO 供体硝酸盐通过间隔物共价连接形成,在体内经过代谢转化为脱乙酰化和/或脱硝基的代谢产物,直接不可逆抑制 COX-1 的活性[78]。NCX4016 具有阿司匹林的所有优点,而且还兼具 NO 供体药物的特点(抗血栓形成、抗动脉硬化和扩张血管等)。实验已经证实了 NCX4016 对再狭窄[79]和静脉移植后的糖尿病患者都有良好的血管扩张作用[80]。

AA 转化生成的 TXA_2 和血小板活化后释放的 TXA_2 都是通过作用于 TP 受体发挥作用,抑制了 TP 受体,TXA_2、AA、内过氧化物等的作用都将被抑制,这种作用可能比阿司匹林抑制 COX 所达到的血小板抑制效果更强。特鲁曲班(terutroban)是前几年新研发的口服 TP 受体拮抗剂,在提高血管内皮细胞功能的同时具有抗动脉粥样硬化的功效。大量动物和临床实验都表明,特鲁曲班在预防动脉粥样硬化,特别是脑血管及心血管领域的卒中和冠状动脉疾病治疗方面潜力巨大[81]。利多格雷(ridogrel)除了抑制 TX 合成酶活性外,也兼具 TP 受

体拮抗剂性质,通过拮抗内过氧化物向前列环素的转换增强其抗血小板聚集效果[82]。

其他 TP 受体拮抗药物,都分别有其各自的优点。伊非曲班(ifetroban)能扭转 TXA_2/PGH_2 的作用[83]。雷马曲班(ramatroban)不仅能拮抗 TP 受体,也能拮抗 PGD_2 受体[84]。吡考他胺(picotamide)的外周动脉疾病的随机测试结果显示其致死率远低于阿司匹林,在糖尿病的治疗中,能减少微量白蛋白同时抑制颈动脉斑块的生长[85]。此外,最新的研究表明 BM573 能够通过抑制 TP 受体以及 TX 合成酶活性阻止载脂蛋白 E 基因缺陷小鼠的早期动脉粥样硬化病变[86];而 EV-007 作为新研发的 TP 受体和 TX 合成酶抑制剂,其效果优于血小板活化和动脉血管病变双重拮抗剂吡考他胺,在抑制 2 型糖尿病患者血小板聚集方面,效果也优于阿司匹林[87]。AA 通路相关抑制剂见表 3。

Table 3　Properties of arachidonic acid relative blockers

Agent	Class	Formular	Mechanism	Administration	Development	Reference
Aspirin	Non-steroidal anti-inflammatory drug	$C_9H_8O_4$	COX-1 inhibitor irreversible	*po*	Approved	[88]
Indomethacin	Non-steroidal anti-inflammatory drug	$C_{19}H_{16}ClNO_4$	COX-1 inhibitor reversible	*po*	Approved	[89]
NCX4016	NO-aspirin derivative	$C_{16}H_{13}NO_7$	COX-1 inhibitor irreversible	*po*	Phase II	[90]
Terutroban	Tetrahydro non-prostaglandin derivative	$C_{20}H_{22}ClNO_4S$	TP inhibitor, reversible	*po*	Phase III (S18886)	[91]
BM573	Torasemide derivative	$C_{18}H_{22}N_4O_5S$	TX synthase inhibitor TP antagonist	*po*	Preclinical	[86]
Ifetroban		$C_{25}H_{32}N_2O_5$	TP inhibitor, reversible	iv	Approved	[92]
Ramatroban		$C_{21}H_{21}FN_2O_4S$	TP inhibitor, reversible	*po*	Approved	[93]
Ridogrel		$C_{18}H_{17}F_3N_2O_3$	TX synthase inhibitor TP antagonist	*po*	Approved	[94]
Picotamide	Isophthalic acid derivative	$C_{21}H_{20}N_4O_3$	TX synthase inhibitor TP antagonist	*po*	Approved	[85]
EV-077			TX synthase inhibitor TP antagonist	*po*	Phase II	[87]

2.4　PAF 受体拮抗剂

研究表明,PAF 具有多种生物活性,与缺血性心血管疾病、脑缺血、消化系统功能障碍、哮喘、肾脏损害等多个生理病理过程都有密切关系[95]。因此,PAF 拮抗剂在上述疾病治疗中具有重要价值。目前,尚无化学合成 PAF 拮抗剂上市销售,但已有一些化学实体处于积极研究状态(如表 4)。

最早的 PAF 拮抗剂是 CV-3988,属于 PAF 结构类似物,能显著抑制 PAF 诱导的家兔血小板聚集,对 AA、ADP、胶原以及 A-23187 诱导的血小板聚集没有影响,能剂量依赖地抑制 PAF 诱导的大鼠低血压[96]。CV-3988 的优点是不会引起血压、脉搏、呼吸频率等指标变化,可以安全有效的用于人体[97]。

妥洛帕泛(tulopafant)能显著减少巴比妥麻醉狗的心肌梗死面积,降低缺血以及再灌注引发的心律失常的概率[98]。来昔帕泛(lexipafant)是已知具有最强特异性的 PAF 拮抗剂之一,急性胰腺炎早期给予 PAF 拮抗剂来昔帕泛治疗时,可使并发症减少,病死率降低,该药极大可能成为治疗急性胰腺炎的首个上市药品[99]。阿帕芬(apafant)是选择性 PAF 受体拮抗剂,在 101 位健康志愿者的实验中证实没有明显的副作用,获得良好的药代动力学(线性)和药理学参数,临床实验中用于治疗哮喘[100]。依拉帕泛(israpafant)在体内能抑制皮肤过敏性反应,包括嗜酸粒细胞增多[101]以及呼吸道过敏性反应(微血管渗漏[102]、支气管收缩[103]等)。肾小球血栓模型鼠注射 PAF 拮抗剂 SM-12502,能减少肾小球纤维素沉积[104]。

Table 4　Properties of platelet activating factor (PAF) receptor antagonists

Agent	Class	Formular	Indication	Administration	Development	Reference
CV-3988	PAF analogs	$C_{28}H_{53}N_2O_7PS$	Research tools	iv	Reaserch Tool	[96]
Tulopafant		$C_{25}H_{19}N_3O_2S$	Anticoagulant thrombolytic drugs	*po*	Phase I(RP59227)	[98]
Lexipafant		$C_{23}H_{30}N_4O_4S$	Acute pancreatitis	iv	Phase III(BB-882)	[105]
Apafant	Thiophene and triazole benzodiazepines	$C_{22}H_{22}N_5O_2SCl$	Anti-inflammatoryanti-angiogenic anti-tumor	*po*	WEB 2086	[106]
Israpafant	Thiophene and triazole benzodiazepines	$C_{28}H_{29}ClN_4S$	Anti-allergic anti-asthmatic	*po*	Phase III(Y-24180)	[107]
SM-12502	Pyridine-thiazolidine	$C_{10}H_{12}N_2OS$	Anti-septic shock	iv	Phase II	[108]

2.5　5-HT 受体抑制剂

在诸多 5-HT 受体中,只有 $5\text{-}HT_{2A}$ 与血管平滑肌收缩、血小板聚集、血栓形成及冠状动脉痉挛收缩有关,所以 $5\text{-}HT_{2A}$ 受体抑制剂具有治疗心血管

疾病的潜力(表5)。

Table 5 Properties of 5-hydroxytryptamine (5-HT) relative blockers

Agent	Formular	Mechanism	Administration	Development	Reference
Ketanserin	$C_{22}H_{22}FN_3O_3$	$5HT_{2A}$ antagonist	*po*	Approved	[115]
Ritanserin	$C_{27}H_{25}F_2N_3OS$	$5HT_{2A}$ antagonist	*po*	Approved	[116]
Cyproheptadine	$C_{21}H_{21}N$	Histamine H_1 inhibitor, 5-HT_{2A} inhibitor	*po*	Approved	[110]
Pizotifen	$C_{19}H_{21}NS$	Histamine H_1 inhibitor, 5-HT_{2A} inhibitor	*po*	Approved	[110]
AT-1015	$C_{29}H_{33}N_3O_2$	5-HT_{2A} inhibitor, high selectivity	*po*	Phase II	[111]
Sarpogrelate	$C_{24}H_{31}NO_6$	5-HT_{2A} inhibitor	*po*	Approved	[117]
R96544	$C_{22}H_{29}NO_3$	5-HT_{2A} inhibitor	*po*	Reaserch tool	[113]
AR246686	$C_{24}H_{26}ClBrN_4O_2$	5-HT_{2A} inhibitor, high selectivity	*po*	Preclinical	[118]
SL65.0472	$C_{24}H_{31}N_4O_4S$	Mixed 5-HT_{1B}/5-HT_{2A} receptor antagonist	*po*	Reaserch tool	[119]
APD791	$C_{24}H_{31}N_4O_4$	5-HT_{2A} inhibitor	*po*	Phase I	[120]

酮色林(ketanserin)是最早发现的高亲和力5-HT_{2A}受体抑制剂,长期服用酮色林可以显著降低血压变异性,改善受损的动脉压力反射功能[109]。除了抑制5-HT_{2A}受体以外,酮色林还兼具选择性5-HT_{2C}受体和α-1肾上腺素能受体抑制功能,同时对组胺H_1受体的亲和性非常高,这些受体都具有中央交感属性和直接的血管舒张性质,所以虽然酮色林的降压效果很好,但长期服用却有致心律失常的危险。相比之下,利坦色林(ritanserin)对5-HT_{2A}的选择性比酮色林好。

赛庚啶(cyproheptadine)对血管平滑肌细胞上的5-HT有强烈的阻断作用,与酮色林一样也是组胺H_1受体抑制剂。有研究表明,赛庚啶和苯噻啶(pizotifen)作为抗抑郁症5-HT_{2A}受体抑制剂,同样能发挥抗血小板和抗血栓作用,只是这种作用对正常的血栓形成有一定干扰[110]。

老一代的5-HT受体抑制剂虽然都有抑制5-HT_{2A}的作用,但普遍存在选择性不太专一的缺点。因此,其他改善了受体选择性的药物逐渐被开发。AT-1015是长效5-HT_{2A}高选择性抑制剂,强烈抑制5-HT诱导的血管收缩,也能改善月桂酸引起的大鼠外周血管病变[111]。SL65.0472是5-HT_{1B}/5-HT_{2A}混合抑制剂,对5-HT诱导的血管收缩和血小板聚集有抑制作用[112]。R96544是前药型5-HT_{2A}受体拮抗剂R102444的活性部分,是强效竞争选择性5-HT受体拮抗剂[113]。沙格雷酯(sarpogrelate)也是选择性5-HT_{2A}受体拮抗剂,在临床上作为与血栓形成相关的缺血性疾病治疗药物,分子模拟研究表明,沙格雷酯可能在心血管疾病治疗方面具有良好的药理作用[114]。

2.6 膜糖蛋白受体GPⅡb/Ⅲa拮抗剂

GPⅡb/Ⅲa是血小板聚集的最终途径,而且GPⅡb/Ⅲa受体只存在于血小板上,占血小板蛋白总数的1%~2%,是最大量的血小板表面受体。GPⅡb/Ⅲa受体拮抗剂可以阻止血小板聚集最重要的一部分,其所表现出的抗血小板效果比阿司匹林或者ADP受体拮抗剂的临床评价有力得多。目前临床上应用的GPⅡb/Ⅲa受体拮抗剂(表6)可以分为3类:单克隆抗体、非肽类抑制剂以及合成肽类抑制剂[121]。

Table 6 Properties of glycoprotein GPIIb/IIIa antagonist

Aggent	Class	Formular	Indication	Administration	Development	Reference
Abciximab	Monoclonal antibodies	$C_{2101}H_{3229}N_{551}O_{673}S_{15}$	Assisted percutaneous coronary intervention in the prevention of cardiac ischemic complications	iv	Approved(Reopro®)	[127]
Eptifibatide	Synthetic peptides	$C_{35}H_{49}N_{11}O_9S_2$	Unstable angina, acute myocardial infarction	iv	Approved(Integrilin®)	[128]
Tirofiban	Non-peptide tyrosine derivative	$C_{22}H_{36}N_2O_5S$	Unstable angina or non-Q-wave myocardial infarction	iv	Approved(Aggrastat®)	[124]
Lamifiban	Non-peptidic non-cyclic	$C_{24}H_{28}N_4O_6$	Unstable angina or non-Q-wave myocardial infarction	iv	Phase III	[126]

阿昔单抗(abciximab)是第1个GPⅡb/Ⅲa受体拮抗剂,它是一种人-鼠嵌合的大分子单克隆抗体(嵌合抗原结合片段的小鼠抗人GPⅡb/Ⅲa受体),与GPⅡb/Ⅲa非特异性结合后,能改变受体

空间结构,导致纤维蛋白原无法结合。但阿昔单抗由于具有潜在的免疫原性,在运用中易出现过敏出血反应[122]。

模仿 GPⅡb/Ⅲa 受体上发现的精氨酸-甘氨酸-天冬氨酸(RGD)序列,将依替巴肽(eptifibatide)合成为活性基团为赖氨酸-甘氨酸-天冬氨酸(KGD)的环状七肽,以此阻断 GPⅡb/Ⅲa 与凝血因子结合。依替巴肽与 GPⅡb/Ⅲa 受体结合的两个必须条件是 Ca^{2+} 和完整的受体结构,这与生理配体的结合机制很相似。所有小分子的拮抗剂生物半衰期和对 GPⅡb/Ⅲa 的亲和力都低于阿昔单抗。依替巴肽与受体的亲和力没有阿昔单抗高,循环血液中的大部分依替巴肽都没有与血小板结合,当静脉注射停止后,血小板的功能就会恢复,这种可逆的抑制作用容易引发并发症[123]。

替罗非班(tirofiban)是由酪氨酸类似物优化得到的小分子非肽类拮抗剂,结构上模仿了去整合素蛇毒锯鳞蝰的 RGD 环[124],与受体结合速率很快[125]。与依替巴肽类似,其作用效果也具有快速及可逆性。1998 年 FDA 批准其在美国上市,也是我国第 1 个血小板 GPⅡb/Ⅲa 受体拮抗剂。拉米非班(lamifiban)是合成的非环状非肽类的低分子化合物,临床应用时容易出现出血并发症[126],阿斯利康在Ⅲ期临床实验后终止了研究。

2.7　PDE 抑制剂

cAMP 和 cGMP 是两个重要的调节血小板功能的抑制性细胞内第二信使,磷酸二酯酶(phosphodiesterases,PDEs),能催化 cAMP 及 cGMP 水解生成失活的 5′-AMP 和 5′-GMP,抑制细胞内环核苷酸水平,从而调节环核苷酸信号的幅度、持续时间及间隔[129]。因此,抑制 PDEs 可能会导致很强的血小板抑制作用。血小板具备 3 个 PDE 亚型(PDE2,PDE3,PDE5),不同的亚型对 cAMP 和 cGMP 具有不同的选择性。目前已经开发出数个非选择性或具有同功酶选择性的 PDE 抑制剂,其中有一些作为抗血小板药物已经进入临床使用(表 7)。

Table 7　Properties of PDE inhibitor

Agent	Formular	Mechanism	Indication	Administration	Development	Reference
Pentoxifylline	$C_{13}H_{18}N_4O_3$	PDE inhibitor non-selective	Vasoactive drugs in patients with intermittent claudication non-specific peripheral vasodilator	*po*	Approved	[140]
EHNA	$C_{14}H_{14}ClN_5O$	PDE2 inhibitor	Research tools	*po*	Research tool	[132]
Anagrelide	$C_{10}H_7Cl_2N_3O$	PDE3 inhibitor potent broad-spectrum	Idiopathic thrombocythemia and polycythemia vera concurrent thrombocythemia	*po*	Approved	[135]
Cilostazol	$C_{20}H_{27}N_5O_2$	PDE3 inhibitor potent,specific	Peripheral vascular disease in patients with intermittent claudication	*po*	Approved	[141]
Milrinone	$C_{12}H_9N_3O$	PDE3A inhibitor potent,specific	Acute and chronic refractory congestive heart failure	*po*,iv	Approved	[142]
Dipyridamole	$C_{24}H_{40}N_8O_4$	PDE3,PDE5 inhibitor	Coronary vasodilator	*po*,iv	Approved	[143]

己酮可可碱(pentoxifylline)能降低全血黏度,改善红细胞的变形能力,通过 PGI_2 增强体内抑制血小板作用,抗血小板活性很强[130],在体外能够以高浓度抑制全血中的血小板聚集[131]。

PDE2 亚型能同时水解 cAMP 及 cGMP 两种环核苷酸,EHNA 兼具中等效力 PDE2 抑制作用和腺苷脱氨酶强抑制效力,EHNA(20 μmol/L)对血小板聚集没有直接作用,但它可通过硝普钠(一种鸟苷酸环化酶刺激物)增强凝血酶诱导血小板聚集的抑制作用[132]。PDE2 抑制剂主要被用作研究工具,目前的研究旨在评价其对记忆障碍和炎症内皮细胞通透性预防的有效性[129]。

PDE3 亚型包括两个亚科,PDE3A 和 PDE3B,而 PDE3A 是 PDE3 在血小板中表达的主要亚科[133]。阿那格雷(aragrelide)是 PDE3 的强抑制剂,也是血小板聚集的一种有效的广谱抑制剂(IC_{50} = 36 nmol/L)[134],尽管阿那格雷抑制巨核细胞成熟的机制尚不完全明确,但该药物已进入临床,用于患者的原发性血小板增多症[135];西洛他唑(cilostazol)是血小板和平滑肌细胞内 PDE3 的特异性强效抑制剂(IC_{50} = 0.2 μmol/L),它可以降低细胞内 Ca^{2+} 浓度,松弛平滑肌细胞,抑制血小板活化[136];米力农(milrinone)是一种特定的 PDE3A 抑制剂,呈剂量依赖性引起血小板内 cAMP 升高,从而抑制血小板聚集,目前用于充血性心脏衰竭临床治疗[137]。

双嘧达莫(dipyridamole)是 PDE5 和 PDE3 双重抑制剂,通过以下几种途径影响血小板功能:抑

制红细胞对腺苷的重吸收,提高血管中的血浆以及血小板抑制性核苷酸水平;显著提高血小板内cAMP和/或cGMP浓度;清除自由基灭活环氧化酶,促进PGI_2合成。双嘧达莫已经合成超过50年,最初用作冠状血管扩张药,后来发现双嘧达莫能抑制家兔体内血小板聚集和血栓形成,因此作为抗血小板药用于心脏手术或瓣膜置换术[138-139]。

2.8 其 他

除上述抗血小板药物外,还有一些作用于血小板活化通路中其他位点的药物(表8),这些药物现大多处于研究阶段,它们也为更好的治疗血栓性疾病提供可能性。

Table 8 Other antiplatelet compounds

Agent	Class	Mechanism	Reference
Kistomin	Venom extract	GPVI antagonist	[159]
Crotalin	Venom extract	GPIb antagonist	[147]
Revacept	Venom extract	GPVI antagonist	[146,160]
DZ-697b	New Structure	Adhesion antagonist	[148]
DG-041	Bis sulfonyl sulfonamides	EP3 inhibitor	[161]
TGX-221	Morpholino-pyrimidone	PI3K inhibitor	[158]
PSI-697	Quinoline salicylic	P-selectin inhibitor	[154]
PSI-421	Quinoline-carboxylic acid	P-selectin inhibitor	[156]
Bimosiamose	Sugar analogs	P-selectin inhibitor	[162]
rPSGL-1-Ig	Recombinant immunoglobulins chimeras	P-selectin inhibitor	[156]

2.8.1 *黏附拮抗剂* 血小板膜糖蛋白GPⅥ和GPⅠb在血小板黏附到内皮下基质过程中起到了至关重要的作用[144]。Kistomin是一种蛇毒金属蛋白酶(SVMPs),能够裂解血小板膜糖蛋白Ⅵ[145],从而阻断胶原与GPⅥ受体的反应,为新抗血栓药物的开发提供选择。Revacept是人GPⅥ-Fc融合蛋白,能阻止血小板在血管损伤部位激活,作用类似于"血管涂层"。研究表明,Revacept能显著减少动脉血管斑块上的血栓形成并且对凝血动态平衡没有影响[146]。Crotalin是从响尾蛇蛇毒中分离出的一种膜糖蛋白GPⅠb受体拮抗剂,能够阻止vWF与GPⅠb相互反应,阻止体内血小板栓子的形成[147]。DZ-697b在人体体外实验中选择性抑制胶原和vWF引起的血小板聚集[148]。

2.8.2 *EP3前列腺素PGE_2受体抑制剂* PGE_2是在动脉粥样硬化斑块形成时产生的炎性介质,EP3是PGE_2的受体,与ADP受体P_2Y_1类似,可以抑制cAMP的生成,但不会引发Ca^{2+}动员[149]。EP3受体拮抗剂可能提供动脉粥样硬化病变的特异性抗血栓治疗。DG-041是直接EP3抑制剂,能够为P_2Y_{12}受体拮抗剂增效[150],在治疗动脉粥样硬化的同时不会引起不必要的出血[151]。

2.8.3 *P-选择素抑制剂* P-选择素只在活化的内皮细胞和活化的血小板上表达,这使得P-选择素成为一个主要的检测指标[152]。P-选择素糖蛋白配体1(P-selecting lycoprotein ligand,PSGL-1)在白细胞表面表达,血小板活化后,致密颗粒分泌释放P-选择素,当它与PSGL-1作用后,使白细胞、血小板、内皮细胞之间的活动增加[153],促进更多血小板聚集到内皮细胞表面,引起血管闭塞。所以,阻止P-选择素与PSGL-1之间的相互作用具有心血管疾病的治疗潜力。

PSI-697是基于C-2苄基取代的喹啉水杨酸P-选择素抑制剂,在多种心血管疾病的动物模型中都很有效,一阶段的单剂量递增研究中显示出良好的耐受性,但是口服生物活性比较低[154]。PSI-421是在保留PSI-697效力的情况下,改变其部分结构,增加药物溶解度,改善药物部分动力学性质得到的新化合物[155],这种化合物具有动静脉损伤动物模型的口服疗效,被选定为动脉粥样硬化和深静脉血栓形成治疗的临床前开发复合物[156]。另外,P-选择素抑制剂Bimosiamoser和PSGL-1-Ig也已经进入临床实验阶段[156-157]。

2.8.4 *PI3K抑制剂* PI3K是整合素αIIbβ3介导的血小板活化通路中的关键因子,TGX-221是具有细胞渗透性的可逆ATP竞争性PI3K激酶抑制剂,抑制剪切力条件下的血小板活化,并且不会延长出血时间[158]。

3 结 语

血小板已经成为生理血管修复及其病理紊乱

的关键细胞[163],是血栓形成,炎症反应以及动脉粥样硬化之间联系的关键环节。血小板可以经由 THR,TXA_2,ADP 等多种诱导剂通过不同的通路激活,不受控的血小板活化会形成富血小板血栓,导致心肌梗死,缺血性卒中等临床缺血事件。目前的抗血小板化学药,比如凝血酶抑制剂、ADP 受体拮抗剂、GPⅡb/Ⅲa 受体抑制剂等,在动脉粥样硬化疾病治疗方面疗效显著。但是这些药物,特别是在联用的时候,会增加出血风险,而其药物抵抗效应可能与血小板介导血栓形成的非全面抑制有关。在进一步的研究中:一是优化现有的药物,降低药物不良反应或者抵抗性,优化多种不同作用机制的抗血小板药的联用配比,同时积极寻找新靶点;二是利用传统活血化瘀中药抗血小板聚集的多靶点多途径的优势[164],采用中西药结合的手段治疗血栓性疾病。课题组对近年来血小板在活血化瘀中药研究方面的应用进行了总结[164],表明针对活血化瘀中药的抗血小板作用研究是该类中药研究的重点之一。近期本课题组针对 31 种活血化瘀中药的不同极性提取物进行了体外抗血小板聚集活性评价[165],发现大多数中药提取物具有良好的抗血小板聚集活性,表明抗血小板聚集是该类中药发挥活血化瘀的重要途径。进一步开展抗血小板聚集活性高的中药的作用机制研究,并采用生物色谱等手段筛选其中的活性成分;研究不同活性成分的作用机制及靶点,寻找中药活性成分间的协同或拮抗作用是课题组的长期研究方向之一。

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