

甚为重要, 血小板破裂后放出凝血致活酶元酶, 能活化血浆中凝血致活酶元——即凝血致活酶的前身⁽⁹⁾。本文实验观察到凝血酶时间延长, 山萘苕碱阻止凝血酶的生成, 即能阻止血小板凝集, 亦能阻止血栓形成。此外, 当纤维蛋白降解产物增加时, 凝血酶时间延长, 本实验结果与此相符, 提示山萘苕碱有一定的促纤溶作用。

综上所述, 山萘苕碱抑制血栓作用与血小板功能及纤维蛋白有关, 可抗红、白两种血栓的形成, 改善血液流变性。

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刺参酸性粘多糖与洗涤的人血小板的结合¹

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Binding of acid mucopolysaccharides of *Stichopus japonicus* to washed human platelets¹

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ABSTRACT The binding of acid mucopolysaccharides of *Stichopus japonicus* Selenka (Sjamp) to washed human platelets was studied using [³H]Sjamp. The binding was rapid, reversible and with a saturable tendency. The binding reached to equilibrium at about 10 min, and 50% of [³H]Sjamp bound to the platelets at equilibrium within the first minute. When excess unlabeled Sjamp was added to the [³H]

Sjamp binding suspension, [³H]Sjamp dissociated from the platelets rapidly and rather completely. Scatchard analysis revealed a class of binding sites with $K_d = 2.3 \pm 0.7 \mu\text{g}/\text{ml}$ and $B_{max} = 4.5 \pm 1.2 \mu\text{g}/10^9$ platelets. Separate experiments showed that the monoclonal antibody (McAb) SZ-21, a McAb to platelet membrane glycoprotein (GP) III_b, weakened the binding, while McAb SZ-2 and SZ-22 (to GP I_b and GP II_b respectively) were ineffective. We also observed that heparin inhibited [³H]Sjamp binding to the platelets. These results indicated that the binding sites on platelets might be the Sjamp receptors. We conclude that receptor-Sjamp binding is an important process in platelet aggregation induced by Sjamp.

KEY WORDS *Stichopus japonicus*; mucopolysaccharides; binding sites; blood platelets; radio-ligand assay; monoclonal antibodies; heparin

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摘要 用氚标刺参酸性粘多糖(^3H]Sjamp)与洗涤的人血小板的结合实验,发现Sjamp结合于血小板上,该结合有下列特点:(1)1 min内结合迅速,10 min达到平衡;(2)结合具有可逆性;(3)Scatchard分析得出结合点的 $K_d=2.3\pm\text{SD }0.7\ \mu\text{g/ml}$ ($n=4$), $B_{\text{max}}=4.5\pm 1.2\ \mu\text{g}/10^9$ 血小板;(4)McAb SZ-21(抗GP III_a)能减少Sjamp的结合,该结合能被肝素抑制。本文认为血小板上存在Sjamp受体。

关键词 刺参;粘多糖;结合位点;血小板;放射配位体测定;单克隆抗体;肝素

刺参(*Stichopus japonicus* Selenka)酸性粘多糖(acid mucopolysaccharide, Sjamp)是从棘皮动物刺参体壁提取的一种硫酸粘多糖⁽¹⁾。Sjamp具有抗凝血和诱导血小板聚集的作用^(2,3),用Sjamp作血小板诱聚剂,曾发现新型血小板聚集功能缺陷症⁽⁴⁾。本文用 ^3H]Sjamp作为放射配体对16例健康人血小板进行放射结合实验,发现 ^3H]Sjamp结合于生理盐水洗涤的人血小板。初步认为血小板上存在Sjamp受体,并与Sjamp诱导血小板聚集有关。

MATERIALS AND METHODS

Sjamp (10 mg/ml)由天津医药科学研究所提供, ^3H]Sjamp为中国原子能研究院同位素研究所标记,比放射性为24 MBq/mg,放射浓度为29 MBq/ml。肝素(12 500 IU/ml,上海生物化学制药厂)。单克隆抗体(McAb)SZ-2, SZ-21, SZ-22均由苏州医学院止血与血栓研究室提供。上述试剂在必要时用生理盐水稀释。

洗涤血小板悬液 取健康人静脉血30-50 ml于装有相当于1/9血容量1% EDTA的硅化试管中,迅速混匀,150×g离心10 min。取上清液(PRP)再以1400×g离心12 min,去上清,用等容量生理盐水洗涤2次。再进行离心1400×g,10 min后去上清,用大约一半容量(记具体数值)的生理盐水悬浮血小板,细吸

管轻轻打匀,调整血小板数约为20万/mm³。

^3H]Sjamp与血小板的结合

1 结合量的测定 在96孔板中加入不同浓度的 ^3H]Sjamp和生理盐水各25 μl ,再加入血小板悬液200 μl ,细玻璃棒搅匀,室温(约22℃)下作用规定时间后,用细胞收获器(Titertek cell harvester, USA)将各孔中血小板迅速收集在孔径为0.45 μm 的玻璃纤维滤膜上,蒸馏水冲洗3次,每次10 s,37℃烘干,放进盛有5 ml闪烁液(0.5% PPO, 0.05% POPOP的二甲苯溶液)的瓶中进行放射性测定,所测值为总结合(TB),另应用100倍以上于 ^3H]Sjamp浓度的非标记Sjamp 25 μl 代替上述生理盐水,所测结果为非特异性结合(NSB),TB减去NSB即为特异性结合(SB),实验中设3平行管。

2 Scatchard分析 选择受体Clark模型⁽⁵⁾,应用Scatchard法⁽⁶⁾作图求出血小板Sjamp结合点的 K_d 和 B_{max} 。

3 单克隆抗体对 ^3H]Sjamp结合的影响 在浓度为250 $\mu\text{g/ml}$ 的McAb SZ-2, McAb SZ-22或不同浓度的McAb SZ-21 25 μl 与血小板悬液200 μl 温育(37℃)8 min后加入22.5 $\mu\text{g/ml}$ 的 ^3H]Sjamp 25 μl 搅匀,室温下作用20 min,抽滤以中止反应,测血小板上的 ^3H]Sjamp结合量。设双平行管。

4 肝素对 ^3H]Sjamp结合的影响 在200 μl 血小板悬液中同时加入不同浓度肝素和 ^3H]Sjamp (22.5 $\mu\text{g/ml}$)各25 μl ,作用20 min后中止反应,测血小板上的放射性。实验中设双平行管。

RESULTS

^3H]Sjamp的结合及解离的时间过程 如图Fig 1 A所示,在最初1 min内, ^3H]Sjamp与血小板结合迅速,然后结合速度减慢。至10 min后,随着时间延长,结合量(SB)增加不多,结合基本达到平衡。若平衡时加入高浓度的非标记Sjamp,大部分特异性结合的 ^3H]

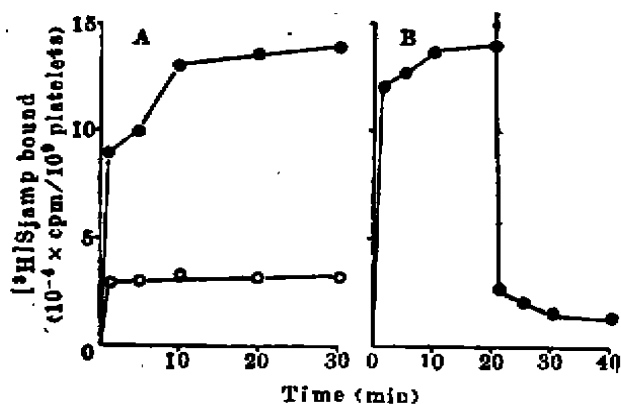


Fig 1. Binding (A) and dissociation (B) of mucopolysaccharides of *Stichopus japonicus* Selenka ($[^3\text{H}]\text{Sjamp}$) 2.25 $\mu\text{g}/\text{ml}$ to platelets. A) $n=4$, (○) nonspecific binding, (●) specific binding. B) Data from one representative experiment are shown. Arrow indicates adding of unlabeled Sjamp (400 $\mu\text{g}/\text{ml}$).

Sjamp 被解离, 且解离过程迅速, 解离程度较彻底 (Fig 1 B).

序列浓度的 $[^3\text{H}]\text{Sjamp}$ 与血小板的结合因 Sjamp 的浓度限制, 没有取得完整的饱和曲线, 但 SB 有饱和趋势 (Fig 2). SB 平均占 TB (SB + NSB) 的 75-79%, NSB 随 $[^3\text{H}]\text{Sjamp}$ 浓度的增加呈线性上升. 作数值转换后 Scatchard 法作图 (Fig 2, inset), 初步求得洗

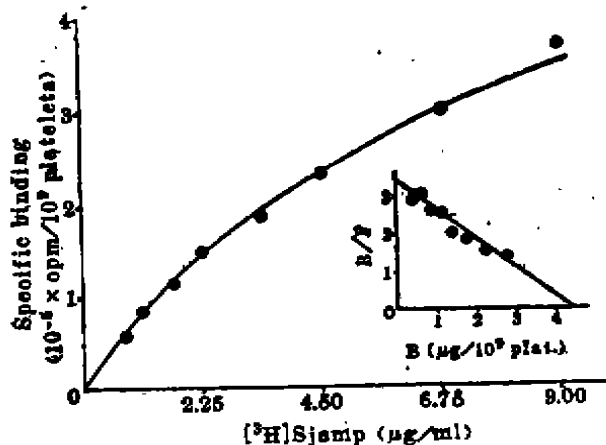


Fig 2. Binding of $[^3\text{H}]\text{Sjamp}$ to platelets and Scatchard analysis (inset). Platelets were incubated with increasing concentrations of $[^3\text{H}]\text{Sjamp}$ (0.84-9.00 $\mu\text{g}/\text{ml}$) for 20 min. $n=4$.

涤的人血小板 Sjamp 结合点的 $K_d = 2.3 \pm 0.7 \mu\text{g}/\text{ml}$, $B_{max} = 4.5 \pm 1.2 \mu\text{g}/10^9$ 血小板.

抗血小板膜糖蛋白单克隆抗体和肝素对 $[^3\text{H}]\text{Sjamp}$ 结合的影响 McAb SZ-2 和 McAb SZ-22 对 $[^3\text{H}]\text{Sjamp}$ 与血小板的结合 (SB) 无明显影响; McAb SZ-21 能减少该结合. 肝素显著抑制 $[^3\text{H}]\text{Sjamp}$ 的结合 (Fig 3). 结果还显示, McAb SZ-21 浓度愈高对结合抑制愈甚. 肝素在浓度为 62.5 IU/ml 时, 对 SB 抑制最明显, 浓度再升高, 抑制程度反而减弱 (Fig 4).

DISCUSSION

近年来, 对血小板上诱聚剂受体的认识有

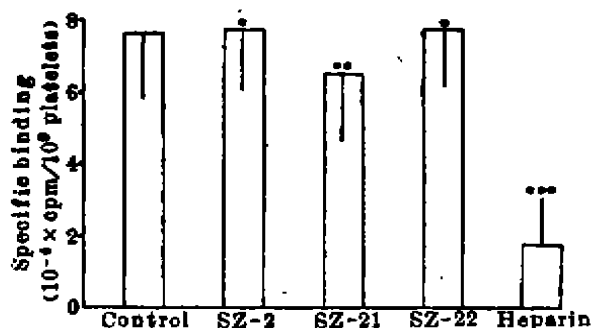


Fig 3. Effects of monoclonal antibody (SZ-2, SZ-21, SZ-22) 25 $\mu\text{g}/\text{ml}$ and heparin 62.5 IU/ml on binding of $[^3\text{H}]\text{Sjamp}$ (2.25 $\mu\text{g}/\text{ml}$) to platelets. $n=8$, $\bar{x} \pm \text{SD}$. * $P > 0.05$, ** $P < 0.05$, *** $P < 0.01$ vs control.

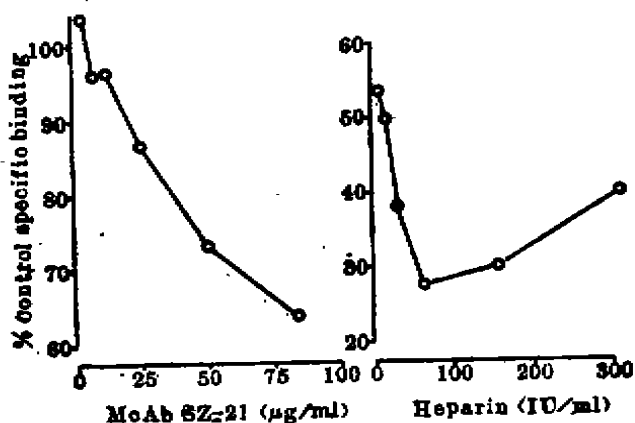


Fig 4. Inhibition of specific $[^3\text{H}]\text{Sjamp}$ (2.25 $\mu\text{g}/\text{ml}$) binding to platelets by McAb SZ-21 and heparin. $n=5$.

了很大进展⁽⁷⁾。Sjamp 作为一种新型血小板诱聚剂，它与血小板结合的特点在本文中进行了探讨。结果显示 [³H]Sjamp 与血小板有一迅速结合的过程，与 Sjamp 诱导血小板聚集曲线上有一短暂的延滞期^(3,8)是一致的；实验说明 (Fig 1) [³H]Sjamp 与血小板的结合是可逆的；随 [³H]Sjamp 浓度增加，它与血小板的结合亦增加 (Fig 2)，与血小板对 Sjamp 聚集反应有剂量依赖关系⁽³⁾ 相符；根据受体 Clark 模型性质⁽⁹⁾，我们认为 K_d 、 $9 \times K_d$ 值 (分别为 2.3 和 20.7 $\mu\text{g/ml}$) 与 Sjamp 诱聚血小板的低限浓度 (2.4 $\mu\text{g/ml}$)⁽³⁾、最适浓度 (25 $\mu\text{g/ml}$)⁽⁸⁾ 是相符的。上述 Sjamp 结合点的特征支持血小板上存在 Sjamp 受体。需要指出的是：本文用经 EDTA 抗凝后洗涤的血小板悬液进行实验；而聚集研究中用枸橼酸钠抗凝的 PRP 作试验，其间的结果会有一些差别。

McAb SZ-21 是一种抗血小板膜糖蛋白 (GP) III_a 的特异性单克隆抗体⁽⁹⁾。本文结果 (Fig 3, Fig 4) 说明血小板 Sjamp 结合点可能 GP III_a 有关，与 GP I_b、GP II_b (单克隆抗体分别为 SZ-2 与 SZ-22^(10,11)) 无明显关系。Sjamp 结合点与 GP III_a 等的确切关系有待进一步研究。肝素与 Sjamp 结构类似⁽¹⁾，有抗凝血和引起血小板聚集的作用⁽¹²⁾。最近有人证实肝素能结合于血小板^(13,14)。我们观察到肝素对 Sjamp 结合的抑制现象，但尚不能区分是竞争或非竞争因素所致。对高浓度肝素对 Sjamp 与血小板结合的抑制作用减弱的机理不清楚。

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