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，山莨菪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，提示山莨菪碱有一定的促纤溶作用。

综上所述，山莨菪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 japanicus* to washed human platelets[1]

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ABSTRACT The binding of acid mucopolysaccharides of *Stichopus japonicus* Selenka (Sjamp) to washed human platelets was studied using [^{3}H]Sjamp. The binding was rapid, reversible and with a saturable tendency. The binding reached to equilibrium at about 10 min, and 50% of [^{3}H]Sjamp bound to the platelets at equilibrium within the first minute. When excess unlabeled Sjamp was added to the [^{3}H]Sjamp binding suspension, [^{3}H]Sjamp dissociated from the platelets rapidly and rather completely. Scatchard analysis revealed a class of binding sites with $K_d = 2.3 \pm 0.7$ μg/ml and $B_{max} = 4.5 \pm 1.2$ μg/10^9 platelets. Separate experiments showed that the monoclonal antibody (McAb) SZ-21, a McAb to platelet membrane glycoprotein (GP) III_a, 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 [^{3}H]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.

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KEY WORDS *Stichopus japonicus*; mucopolysaccharides; binding sites; blood platelets; radioligand assay; monoclonal antibodies; heparin

摘要 用氚标刺参酸性粘多糖([^{3}H]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±SD 0.7 μg/ml(n = 4)，B_{max} = 4.5±1.2 μg/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[^{3}H]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[^{3}H]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，[^{3}H]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/mm^3。

[^{3}H]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[^{3}H]Sjamp和生理盐水各25 μl，再加入血小板悬液200 μl，细玻棒搅匀，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[^{3}H]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[^{3}H]Sjamp结合的影响 在浓度为250 μg/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 μg/ml的[^{3}H]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[^{3}H]Sjamp结合量。设双平行管。

4 肝素对[^{3}H]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[^{3}H]Sjamp (22.5 μg/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

[^{3}H]Sjamp的结合及解离的时间过程 如Fig 1 A所示，在最初1 min内，[^{3}H]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[^{3}H]

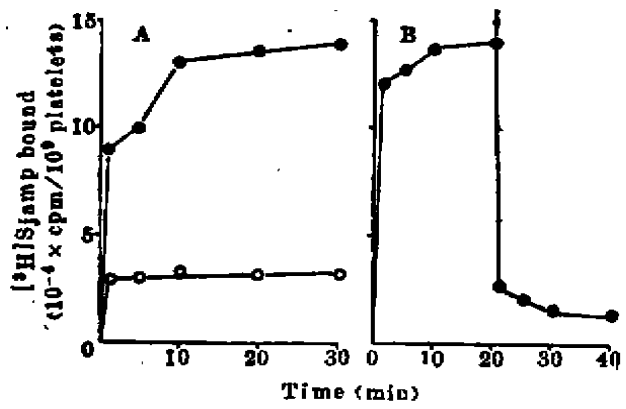

Fig 1. Binding (A) and dissociation (B) of mucopolysaccharides of *Stichopus japonicus* Selenka ([³H]Sjamp) 2.25 μg/ml to platelets. A) $n=4$, (○) nonspecific binding, (●) specific binding. B) Data from one representative experiment are shown. Arrow indicates adding of unlabeled Sjamp (400 μg/ml).

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[³H]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 [³H]Sjamp 浓度的增加呈直线性上升。作数值转换后 Scatchard 法作图(Fig 2, inset)，初步求得洗涤的人血小板 Sjamp 结合点的 $K_d = 2.3 \pm 0.7$ μg/ml，$B_{max} = 4.5 \pm 1.2$ μg/10^9 血小板。

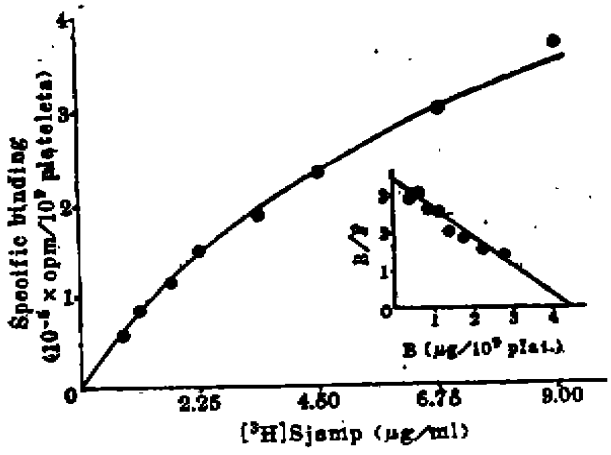

Fig 2. Binding of [³H]Sjamp to platelets and Scatchard analysis (inset). Platelets were incubated with increasing concentrations of [³H]Sjamp (0.84-9.00 μg/ml) for 20 min. $n=4$.

抗血小板膜糖蛋白单克隆抗体和肝素对 [³H]Sjamp 结合的影响 McAb SZ-2 和McAb SZ-22 对[³H]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[³H]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

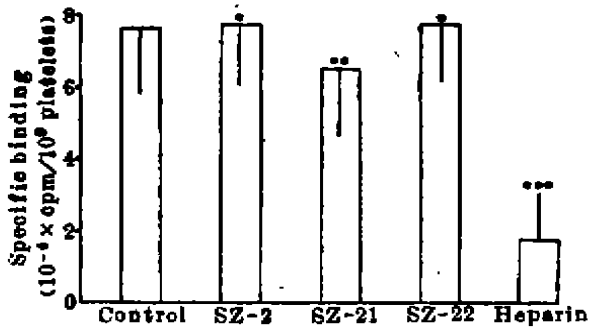

Fig 3. Effects of monoclonal antibody (SZ-2, SZ-21, SZ-22) 25 μg/ml and heparin 62.5 IU/ml on binding of [³H]Sjamp (2.25 μg/ml) to platelets. $n=8$, $\bar{x} \pm SD$. *$P>0.05$, **$P<0.05$, ***$P<0.01$ *vs* control.

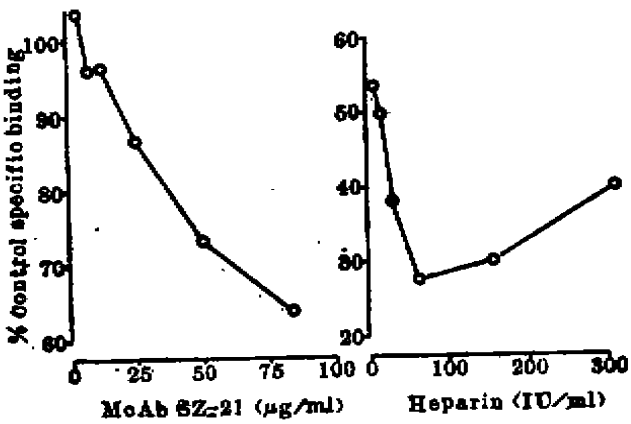

Fig 4. Inhibition of specific [³H]Sjamp (2.25 μg/ml) binding to platelets by McAb SZ-21 and heparin. $n=5$.

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[^{3}H]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)[^{3}H]Sjamp 与血小板的结合是可逆的；随[^{3}H]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[5]，我们认为 K_d、$9\times K_d$ 值(分别为 2.3 和 20.7 μg/ml)与 Sjamp 诱聚血小板的低限浓度(2.4 μg/ml)[3]、最适浓度(25 μg/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人血小板[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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