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Cyclic 3′,5′-Adenosine Monophosphate Level and Adenylate Cyclase Activity in Human Blood Platelets during Storage in ACD Solution

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Summary

The level of cyclic 3′,5′-adenosine monophosphate (cAMP) in human blood platelets and the activity of platelet adenylate cyclase in response to prostaglandin E_1 stimulation do not change during two days storage at room temperature in ACD solution. However, the level of cyclic AMP is lower in platelets stored in ACD solution than in platelets from blood anticoagulated by ethylenediamine tetra-acetic acid.

Zusammenfassung

Die Konzentration von zyklischem AMP und die Aktivität der Adenylat-Zyklase nach Stimulation mit Prostaglandin E_1 in menschlichen Thrombozyten ändert sich nicht, wenn diese zwei Tage bei Zimmertemperatur in ACD-Lösung gelagert werden. Die Konzentration von zyklischem AMP in derart gelagerten Blutplättchen ist niedriger als in Thrombozyten, die aus EDTA ungerinnbar gemachtem Blut gewonnen werden.

Key words: Platelet storage, cAMP, prostaglandin E_1, ACD, EDTA.

Despite the current interest in the biochemistry and pharmacology of platelets [*1*], the level of cyclic 3′,5′-adenosine monophosphate (cAMP) in human blood platelets during their storage for transfusion remains unsettled. Accordingly, we have examined the natural course of cyclic AMP in platelets stored during two days at room temperature in ACD solution and the activity of platelet adenylate cyclase in response to prostaglandin E_1 stimulation during the storage.

A Fenwall blood pack, containing 450 ml whole blood mixed with 67.5 ml ACD solution (0.8% citric acid, 2.2% sodium citrate and 2.24% dextrose) and three Fenwall transfer packs, the first containing 25 ml ACD solution, were used under sterile conditions. The blood pack was centrifuged at 1,700 g for 5 min at 20° C. The resultant platelet rich plasma was further mixed with the ACD solution in the first

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transfer pack and evenly distributed between the three transfer packs. Thus, 270 ml platelet rich plasma, approximately, were mixed with 92.5 ml ACD solution, in accordance with the usual procedure for platelet storage in Fenwall packs. The three transfer packs were stored at room temperature under constant agitation and used for cyclic AMP assay in the platelets on days 0 ($\pm$ 2 h storage), 1 and 2, respectively.

The level of cyclic AMP in the platelets was measured by the method of *Tovey, Oldham* and *Whelan* [*3*], using the assay kit marketed by The Radiochemical Centre, Amersham, England. The platelet rich plasma stored in ACD solution was centrifuged at 4° C, first at 120 g for 15 min to eliminate residual red cells, and again at 27,700 g for 60 min to separate a pellet of platelets from platelet poor plasma. The number of platelets in the pellet was taken as the difference between the count in platelet rich and platelet poor plasma, as determined by the phase microscopy technique. The pellet was re-suspended in 1 ml saline solution, deproteinized with ethanol and extracted as described for plasma samples in the leaflet furnished with the assay kit. Three extracts were prepared from each transfer pack and stored at 4° C until the assay. The results were corrected for recoveries and expressed in terms of picomoles of cyclic AMP per 10^9 platelets.

Day of storage	Platelet concentration (10^9/l) n = 12	Cylic AMP in platelets (pmol/10^9 platelets) n = 36
0 ($\pm$ 2 h)	253 $\pm$ 14	6.7 $\pm$ 0.6
1	246 $\pm$ 14	6.5 $\pm$ 0.6
2	241 $\pm$ 11	5.9 $\pm$ 0.4
	$p > 0.10$	$p > 0.10$

Tab. 1: Level of cyclic AMP in human blood platelets during storage of platelet rich plasma in ACD solution.

Results are from 12 blood donors. Three platelet extracts were assayed for each donor. Data expressed as mean $\pm$ s.e., p by variance analysis.

Platelets obtained from 12 unselected blood donors were stored and assayed. There was a slight but significant increase in the pH of the platelet rich plasma in the transfer packs, from 6.51 $\pm$ 0.03 (s.e.) on day 0 to 6.74 $\pm$ 0.03 (s.e.) on day 2 ($p < 0.001$ by variance analysis). As shown in Tab. 1, the platelet count in platelet rich plasma mixed with the ACD solution and the level of cyclic AMP in the platelets did not change during two days storage ($p > 0.10$ by variance analysis). However, the level of cyclic AMP in the platelets on day 0, or 6.7 $\pm$ 0.6 (s.e.) pmol per 10^9 platelets, was significantly lower ($p < 0.01$) than that measured in platelets separated from blood anticoagulated by ethylene-diamine tetra-acetic acid, i.e. 9.6 $\pm$ 0.6 (s.e.) pmol per 10^9 platelets (49 donors).

Platelets from 10 additional unselected blood donors were examined to evaluate the effect of storage in ACD solution on their responsiveness to prostaglandin E_1 in terms of adenylate cyclase stimulation. The effect of prostaglandin E_1 was tested using the procedure described by *Robison, Arnold,* and *Hartmann* [*2*]. As shown in