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EVALUATION OF A NEW BENZYLSULFONYL-D-ARG-PRO-4-AMIDINOBENZYLAMIDE (BAPA) BLOOD COLLECTION TUBE FOR PLATELET STUDIES

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INTRODUCTION

Benzylsulfonyl-D-Arg-Pro-4-amidinobenzylamide (BAPA) is a potent inhibitor of thrombin and factor Xa. BAPA has previously been described as an alternative to citrate or hirudin anticoagulants for the collection of blood samples to be used for platelet function studies.

AIMS

Using the BAPA tube for T-TAS® 01, to 1) compare the performance of BAPA anticoagulated blood samples to citrate and hirudin anticoagulated blood samples using light transmittance aggregometry (LTA) and T-TAS measurements, respectively, and 2) to determine the stability of BAPA anticoagulated whole blood samples.

METHODS

All blood samples were collected using the BAPA tube for T-TAS 01 or hirudin tubes (Sarstedt and Roche) for T-TAS PL assay measurements using T-TAS Plus, and using sodium citrate for (LTA) measurements.

BAPA and hirudin anticoagulants were compared using T-TAS PL assay measurements from N = 9 matched samples from donors that were either normal healthy donors or were apparently healthy donors taking aspirin. Additionally, a study was performed by adding aspirin *in vitro* to healthy donor blood samples for a total of 25 matched samples with AUC results that span the T-TAS PL assay measurement range.

BAPA and citrate anticoagulants were compared using 22 matched samples from healthy donors and performing LTA measurements using the following agonists: 5 microM adenosine diphosphate (ADP), 2 mM arachidonic acid (AA), 2 microg/mL collagen and 4 microg/mL collagen.

BAPA sample stability was determined using blood samples collected from healthy donors with normal primary hemostatic ability and subjects taking aspirin with abnormal primary hemostatic function. Blood samples were pooled and stored at room temperature prior to testing. Blood samples were stored upright at room temperature for 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, and 24 hours prior to testing. Results within ±15% of the control (t = 30 minute) condition are considered passing.

RESULTS

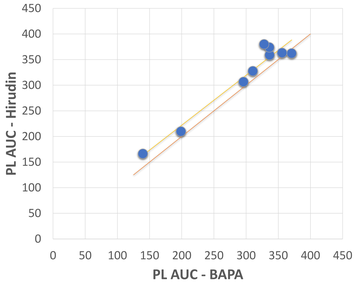
BAPA Sample Stability:

Results were within ±20% of control after 8 hours for all samples and there was no statistically significant slope for AUC (y-axis) vs. time (x-axis).

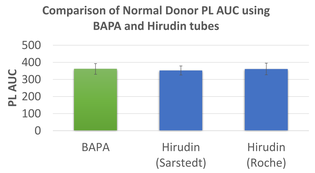
Specimen	Measurement Time	Average PL AUC	% of Control
Normal 1	30min (control)	413.1	--
	1 hour	411.7	99.7%
	2 hours	427.4	103.5%
	4 hours	427.4	103.5%
	6 hours	410.7	99.4%
	8 hours	426.8	103.3%
	24 hours	275.9	66.8%
Normal 2	30min (control)	377.7	--
	1 hour	380.0	100.6%
	2 hours	382.3	101.2%
	4 hours	386.8	102.4%
	6 hours	366.5	97.1%
	8 hours	374.8	99.2%
	24 hours	368.3	97.5%
Abnormal 1	30min (control)	185.7	--
	1 hour	208.0	112.0%
	2 hours	208.3	112.2%
	4 hours	198.6	106.9%
	6 hours	188.5	101.5%
	8 hours	175.7	94.7%
	24 hours	3.7	2.0%
Abnormal 2	30min (control)	189.2	--
	1 hour	188.6	99.7%
	2 hours	211.1	111.5%
	4 hours	195.0	103.1%
	6 hours	192.7	101.9%
	8 hours	158.0	83.5%
	24 hours	161.5	85.4%

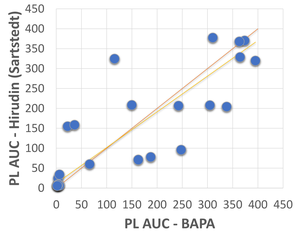
BAPA Comparison with Hirudin:

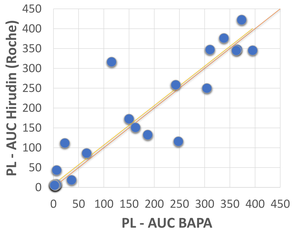
Using native blood samples from normal donors and donors taking aspirin, PL AUC results are highly correlated (r^2 = 0.95) and the Deming regression slope and intercept are 1.00 and -19.3, both not significantly different from 1 and 0, respectively.



Using blood samples from normal donors spiked with different amounts of aspirin, PL AUC results are highly correlated (r = 0.92 for Roche hirudin tubes and r = 0.84 for Sarstedt hirudin tubes) and the slopes and intercepts are not significantly different from 1 and 0, respectively. Variability in results can be attributed to the in vitro manipulation of the samples.







BAPA Comparison with Citrate:

Results from the comparison between BAPA and citrate were evaluated by calculating the average percent recovery according to the formula:

[(BAPA result/citrate result) x 100%]

Parameter	5 µM ADP	2 mM AA	2 µg/mL Collagen	4 µg/mL Collagen
Average Recovery	97.4%	100.8%	100.8%	103.3%
95% Confidence Interval	85.5 – 122.5%	91.3 – 120.8%	83.7 – 119.7%	90.3 – 123.0%

2 donors were later identified as taking aspirin. Both donors had abnormal T-TAS PL assay results and abnormal LTA results using AA and collagen agonists.

CONCLUSIONS

The highly comparable performance and superior sample stability suggest that BAPA is a suitable alternative to citrate and hirudin for anticoagulation of blood samples to be used for platelet function studies.

- Extended sample stability for measuring the platelet thrombus formation process.
- High correlation between BAPA and hirudin with T-TAS PL assay.
  - High correlation observed for native blood samples and contrived blood samples spiked with various amounts of aspirin.
- Comparable recovery between BAPA and citrate with LTA using arachidonic acid, ADP, and collagen.

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CONTACT INFORMATION

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