Stability of urinary thromboxane A_2 metabolites and adaptation of the extraction method to small urine volume

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Background. Thromboxane (TX) A_2 is a pro-thrombotic prostanoid synthesized in activated platelets from arachidonic acid via cyclooxygenase-1 and 2 and TX synthase activities. TXA_2 is unstable (t½: 32 sec) and non-enzymatically converted to the stable, inactive hydration product TXB_2. TXB_2 in humans undergoes hepatic bio-transformation mainly into 11-dehydro-TXB_2, excreted and measurable in urines. Low-dose aspirin inhibits by approx. 70-80% urinary excretion of 11-dehydro-TXB_2 and its recovery after aspirin withdrawal reflects platelets lifespan. Urinary 11-dehydro-TXB_2 level is increased in diseases at high cardiovascular risk and could predict cardiovascular events in aspirin-treated patients. Thus, urinary 11-dehydro-TXB_2 reflects *in vivo* platelets activation and appears a non-invasive, surrogate biomarker of cardiovascular risk and platelet response to antiplatelet drugs. However, this biomarker awaits validation in large prospective trials. A large urine volume (10-8ml in the original method)[1] and the unknown stability of 11-dehydro-TXB_2 in urine after collection are the main methodological difficulties that might lower feasibility and implementation of 11-dehydro-TXB_2 measurement in large clinical trials.

Aims. To adapt the original extraction method from 8 to 1ml urine and assess the stability of 11-dehydro-TXB_2 up to 6 days after urine collection in different experimental conditions.

Methods. Urines were collected from 8 controls, 14 diabetic or 10 non-diabetic patients. We scaled down the original method for 10-8ml[1] to 4, 2 and then 1ml urine sample. The sensitivity of the 1-ml method was tested in aspirin-treated patients.

For stability experiments we measured urinary 11-dehydro-TXB_2 kept in sterile, capped tubes, at 4°C or 25°C 1, 2, 3, 4, 5 and 6 days after collection. The oxidation non-enzymatic product of arachidonic acid, i.e. the 8-iso-prostaglandin (PG)F_{2a}, was also measured to assess oxidative status during the incubation interval. Eleven-dehydro-TXB_2 and 8-iso-PGF_{2a} were measured by enzyme immunoassay (EIA).[2]

Results. Eleven-dehydro-TXB_2 values of 8ml and 1ml extraction methods were highly correlated (rho=0.98,n=33,p<0.001). By Bland-Altman analysis, the mean % difference of [*8ml-1ml*] extraction methods vs absolute 11-dehydro-TXB_2 8ml and 1ml means was -6.6±12%. Deming regression showed no proportional error within the tested concentration range (142pg/mL-2,700pg/mL, regression coefficient=0.002±0.003, p=0.41). In 10 non-diabetic patients fully responsive to aspirin, we could detect in the urine extracts, 11-dehydro-TXB_2 values of 37±32pg/ml (min 10pg/ml).

Eleven-dehydro-TXB_2 values measured in urine incubated at 25°C at each time-point were comparable with and highly correlated to 11-dehydro-TXB_2 values in samples immediately frozen (day 3: 272±175 vs baseline: 300±201pg/ml,n=9,p=0.15; day 6: 505±579 vs baseline: 526±653pg/ml,n=22,p=0.44). No significant differences were found between 11-dehydro-TXB_2 values at baseline, on day 6 at 4°C and 25°C. Since 11-dehydro-TXB_2 excretion is usually corrected for urinary creatinine, we assessed the stability of creatinine and found no significant differences between baseline and day 6 at 25°C (0.83±0.5 vs 0.75±0.5pg/mg,n=10,p=0.73,rho=0.97, p<0.001, at baseline and day 6 respectively). We could not detect any significant *in vitro* generation of 8-iso-PGF_{2a} over the 6 day incubation interval.

Conclusions. Eleven-dehydro-TXB_2 can be measured from small urine volumes and it is relatively stable for few days after collection, even at 25°C. These data might facilitate the validation of this non-invasive, surrogate cardiovascular biomarker in large multicentric studies.

References

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