Stability of urinary thromboxane A₂ metabolites and adaptation of the extraction method to small urine volume

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Background. Thromboxane (TX) A₂ is a pro-thrombotic prostanoid synthesized in activated platelets from arachidonic acid via cyclooxygenase-1 and 2 and TX synthase activities. TXA₂ is unstable (t½: 32 sec) and non-enzymatically converted to the stable, inactive hydration product TXB₂. TXB₂ in humans undergoes hepatic bio-transformation mainly into 11-dehydro-TXB₂, excreted and measurable in urines. Low-dose aspirin inhibits by approx. 70-80% urinary excretion of 11-dehydro-TXB₂ and its recovery after aspirin withdrawal reflects platelets lifespan. Urinary 11-dehydro-TXB₂ level is increased in diseases at high cardiovascular risk and could predict cardiovascular events in aspirin-treated patients. Thus, urinary 11-dehydro-TXB₂ 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-TXB₂ in urine after collection are the main methodological difficulties that might lower feasibility and implementation of 11-dehydro-TXB₂ measurement in large clinical trials.

Aims. To adapt the original extraction method from 8 to 1ml urine and assess the stability of 11-dehydro-TXB₂ 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 10-8ml¹ 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-TXB₂ 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)F₂α, was also measured to assess oxidative status during the incubation interval. Eleven-dehydro-TXB₂ and 8-iso-PGF₂α were measured by enzyme immunoassay (EIA).²

Results. Eleven-dehydro-TXB₂ 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-TXB₂ 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-TXB₂ values of 37±32pg/ml (min 10pg/ml). Eleven-dehydro-TXB₂ values measured in urine incubated at 25°C at each time-point were comparable with and highly correlated to 11-dehydro-TXB₂ 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-TXB₂ values at baseline, on day 6 at 4°C and 25°C. Since 11-dehydro-TXB₂ 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-PGF₂α over the 6 day incubation interval.

Conclusions. Eleven-dehydro-TXB₂ 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