

We assessed urinary levels of 8-iso-PGF2 $\alpha$  and 11-dehydro-TXB2 using developed and validated radioimmunoassay (RIA) techniques<sup>1,2</sup> in part of the Health ABC participants. Gas Chromatography/Mass Spectroscopy (GC/MS) is the most widely used and accepted method for measuring these markers. However, it presents the disadvantage of being onerous and costly that makes it suboptimal for large epidemiologic studies. The excellent reliability and the substantially less costly procedure made the RIA technique preferable for the purpose of our project. This method has been previously used in several studies<sup>3-14</sup>.

Urinary levels of 8-iso-PGF2 $\alpha$  and 11-dehydro-TXB2 were measured at the Laboratory of Clinical Pharmacology of Eicosanoids and Pharmacodynamic located in the Center of Excellence on Aging at the "Gabriele D'Annunzio" University Foundation (Chieti, Italy). For recovery evaluation, 6,000 dpm of [3H]-TXB2 were added to 10 ml of urine samples. After adjustment of the urine pH to 4.0-4.5 with formic acid, 8-iso-PGF2 $\alpha$  and 11-dehydro-TXB2 were extracted on Sep-Pack C18 cartridges (Waters Associates), that had previously been conditioned by washing with 10 ml methanol and 10 ml water. The urine samples were applied to the cartridges and polar material was removed by washing the cartridges first, with 10 ml water and, then with 10 ml hexane. 8-iso-PGF2 $\alpha$  and 11-dehydro-TXB2 were eluted with 10 ml ethyl acetate<sup>1,15</sup>. After evaporation of ethyl acetate to dryness, the extracts were reconstituted with 0.2 ml of benzene/ethyl acetate/methanol (60/40/10, vol/vol) and 0.8 ml of benzene/ethyl acetate (60/40, vol/vol), and finally subjected to silica column chromatography, that had previously been constituted, first with 5 ml benzene/ethyl acetate/methanol (60/40/10, vol/vol) and then, with 5 ml benzene/ethyl acetate (60/40, vol/vol). After applying the reconstituted extracts, the loaded silica columns were washed with 5 ml benzene/ethyl acetate (60/40, vol/vol) and then, finally, 8-iso-PGF2 $\alpha$  and 11-dehydro-TXB2 were eluted with 5 ml benzene/ethyl acetate/methanol (60/40/30, vol/vol). The eluates were dried and reconstituted with phosphate (0.02M, pH 7.4) or Tris-phosphate (0.02M, pH 9.25) buffer for 8-iso-PGF2 $\alpha$  and 11-dehydro-TXB2, respectively, and then analyzed by validated RIA techniques<sup>1,2,15</sup>.

### **RIA techniques**

We developed a validated and reliable RIA for measuring urinary concentrations of 8-iso-PGF2 $\alpha$  by raising antibodies against this compound<sup>2</sup>. In this validation study, urine samples were collected

from 19 healthy subjects (12 males, age range 12-54 yrs). Another group of 20 healthy volunteers (non smokers, aged 21-77 yrs) was also assessed to investigate the influence of increasing age. Overnight urines were collected in the morning and stored at -70°C until analysis. Urine samples were fractionated in aliquots with antioxidant and EDTA. Results from 8-epi-PGF2 $\alpha$  analyses did not show any difference over time. In fact, analysis of 8-epi-PGF2 $\alpha$  over a 6-month period did not show any variation of the levels, suggesting that under these conditions, no spontaneous formation or degradation of this compound occurred. The antisera presented high titers (>1:300,000) and provided highly sensitive assays (IC50: 24 pg/mL). The intra-assay and inter-assay coefficients of variation were  $\pm$ 2.0% and  $\pm$ 2.9% at the lowest level of standard (2 pg/ml) and  $\pm$ 3.7% and  $\pm$ 10.8% at the highest level of standard (250 pg/ml), respectively. Cross-reactivity with other prostaglandin was negligible (7.7% with 8-epi-PGE2, 0.24% with PGF2 $\alpha$ , <0.02% with TXB2, <0.02% with 6-keto-PGF1 $\alpha$ , 0.56% with PGE2, <0.01% with 2,3-dinor-6-keto-PGF1 $\alpha$ , <0.01% with 2,3-dinor-TXB2, <0.01% with 6,15-diketo-PGF1 $\alpha$ ). Different samples of urine were analyzed by GC/MS and the same samples were quantified by enzyme immunoassay. An excellent correlation between the two methods was obtained ( $r=0.99$ ,  $n=9$ ,  $p<0.001$ ). Additionally, 12 urine samples were extracted and purified for RIA. The urine extracts were quantified by RIA using two antisera with a slight difference in cross-reactivities. Similar values were obtained using either antiserum ( $r=0.99$ ,  $n=12$ ,  $p<0.001$ ). In the same study, we also tested the effects of a non-steroidal anti-inflammatory drug (aspirin) on the urinary excretion of 8-epi-PGF2 $\alpha$  and 11-dehydro-TXB2. Single oral dosing with 1 g of aspirin was not associated with any statistically significant variation in the urinary excretion of 8-epi-PGF2 $\alpha$ . On the other hand, urinary excretion of 11-dehydro-TXB2 was inhibited by more than 80%, reflecting the effectiveness of aspirin on its enzymatic target.

The RIA developed for the measurement of 11-dehydro-TXB2 has been shown to be reliable, as well<sup>1</sup>. This method detects changes in the urinary excretion of 11-dehydro-TXB2 associated with simulated short-term increases of TXB2 secretion in human circulation. In fact, 30-minute TXB2 infusions at 5 ng per kg per minute were received by two healthy volunteers, while being on aspirin (325 mg/day) in order to minimize the contribution of endogenous TXB2 production to the measurement of urinary 11-dehydro-TXB2. Urinary excretion of 11-dehydro-TXB2 increased from 5.8

ng/h during the first 2 hours preceding the infusion to 185 ng/h during the 2 hours following the beginning of the infusion. A qualitatively similar pattern was seen during 10-minute infusion of TXB2 at the same rate. A highly significant correlation was reported between the two sets of measurements ( $r=0.975$ ,  $n=8$ ,  $p<0.001$ ). Finally, urinary measurements performed with this method were compared to GC/MS determinations, reporting a highly significant correlation ( $r=0.958$ ,  $n=15$ ,  $p<0.001$ ). The cross-reactivities with 2,3-dinor-TXB2, TXB2, and 11,15-diketo-TXB2 were 0.1%, 0.006%, and 0.002%, respectively. It might be argued that urinary levels of 11-dehydro-TXB2, a platelet activation marker, might be influenced by the platelet count. However, a lack of statistically significant correlation between urinary excretion of 11-dehydro-TXB2 and platelet count ( $r=0.055$ ) has been shown in patients with polycythemia vera<sup>5</sup>.

## References

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