EUROTOX

FEDERATION OF EUROPEAN TOXICOLOGISTS & EUROPEAN SOCIETIES OF TOXICOLOGY

EARLY CAREER AWARDS

Presented during the annual EUROTOX congress to early career scientists judged to have made the best oral or poster presentation. The first and presenting author must be under 35 years of age as of 31 December of the year of the EUROTOX congress.

ECETOC Christa Hennes Early Career Award for toxicological research into mechanisms and risk assessment presented to Dr. Wael Naboulsi at the EUROTOX Brussels congress, September 2-5, 2018.

P23-19: A classical and an immunoaffinity-proteomic study to identify and validate drug-induced kidney injury biomarker in canine

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Canines remain one of the most common non-rodent species used in pre-clinical safety studies. Drug-induced kidney injury (DIKI) is still one of the major reasons for failure in drug development. Not many data about proteome changes in DIKI in canines is available. Here, we conducted a three phases proteomic experiment to high- light the proteome changes in DIKI and to verify some biomarkers known from rat and human studies. In a 10-day (D) low dose-study, animals received the nephrotoxic tobramycin (60 mg/kg, n = 6) or vehicle (n = 3). Minimal to moderate proximal tubular injury was histologically confirmed. First, a discovery proteomic study, we performed a label-free (LF) quantification on the collected kid- ney tissue samples. 558 proteins were significantly differentially regulated (q-value < 0.05) between tobramycin-treated canines and vehicles. Based on the function of the regulated proteins, 12 pro- teins were selected further for analysis. These included proteins which are suggested as DIKI-biomarker such as osteopontin, clus- terin and kidney injury molecule 1 (KIM-1) and unknown proteins such as Serpin A5 and Fatty acid-binding protein. Second, we devel- oped a peptide-centric mass spectrometry-based immunoassay panel (IP-LC/MS) to quantify the candidates in the kidney cortex and in canine's urine. In the IP-LC/MS assay, targeted peptides are enriched by antibodies which recognize a short epitope motif. In accordance with the LF experiment, 8 proteins were found to be significantly up-regulated via the IP-LC/MS assay following the treatment. KIM-1 was mostly affected with 50-fold higher in the treated dogs. Finally, we further validated the IP-LC/MS assay (assay linearity, digestion kinetics, intra- and inter variation) to absolutely quantify the biomarker candidates in the dog urine. Upregula- tion of urinary osteopontin, clusterin, retinol binding protein 4, KIM-1 and protein alpha-1-microglobulin/bikunin on D7 and D10 versus controls was demonstrated while no changes in the stan- dard parameters such as serum creatinine and urea nitrogen were detected. This indicate the applicability of the IP-LC/MS assay to detect changes in urine-based proximal tubular injury biomarkers in canines. Utilizing our short epitope motif enrichment strategy, the developed assay can be applied in monkey, human, mouse, rat and cat studies.