SHORT COMMUNICATION

CHARACTERIZATION OF ALKALINE PHOSPHATASE IN MICE

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Determinations of human serum alkaline phosphatase (ALP) have widespread clinical utility in differential diagnosis and are also used frequently for monitoring treatment. Measurements of mouse ALP is frequently used as a biochemical marker in bone and mineral metabolic research, however; little is known about the circulating ALPs in mice and their association with the human ALP isozymes and isoforms. This study was primarily designed to characterize the circulating and tissue-derived ALP isozymes and isoforms in mice. Tissues from liver, kidney, intestine, and bones from vertebrae, femur and calvaria were obtained and extracted from 20 female mice. Intestine, separated in 7 segments, were collected from 8 wild-type mice and serum was collected from different ALP knockout mouse models: Akp2+/–, Akp2–/– (Tissue non-specific ALP), Akp3–/– (duodenum-specific intestinal ALP). ALP isozymes and isoforms were detected and quantified by high-performance liquid chromatography (HPLC). ALP histochemical staining was performed on mouse liver tissue preparations. Glycosylation differences were studied with Concanavalin A (Con A), Wheat-germ agglutinin (WGA) and Peanut agglutinin (PNA). HPLC analysis of serum from wild-type and knockout mouse models revealed two intestinal fractions, one early eluting peak (Akp3) and ne later peak (Akp6). The same bone ALP isoforms (B/I, B1 and B2) were found in mouse and human serum. In mice, it was also possible to detect the B1x isoform in serum that previously only has been detected in patients with chronic kidney disease and in human bone tissue. It was possible to extract ALP from all tissues except from liver. Histochemical staining confirmed that mouse liver does not contain ALP. HPLC analysis of tissue extracts from kidney showed only one early peak at the void volume and no later peaks. All three bone extracts contained all four bone is forms. ALP activities were found in all intestinal segments with the highest activities in segment 1 and lowest in segment 6 and 7. The first intestinal segment (duodenum) contained three different fractions. Intestinal segments 2, 3 and 4 had one major peak. The tissue extracts showed different migration patterns through the native gel. The native gel demonstrated that all bone tissue samples contained more terminal sialic acid residues than the kidney and intestinal samples. Dose-dependent lectin precipitations demonstrated different glycosylation patterns between the various mouse ALP isozymes and isoforms. The main source of ALP in the human circulation originates from bone, liver and a small fraction intestinal ALP (<5%). In mice we could not extract any ALP from liver, which demonstrates that measuring ALP in mice is not informative when studying liver function. Mouse serum consists of approximately 75% bone ALP, including all four isoforms, B/I, B1x, B1 and B2, and 25% intestinal ALP, which is in agreement with the wild-type mouse serum HPLC profile.