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Blood 142 (2023) 2600–2601

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

321.COAGULATION AND FIBRINOLYSIS: BASIC AND TRANSLATIONAL

Analysis and Mechanism(s) of Hemophilia a Pain in Factor VIII Knockout Mice

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Hemophilia A (HA) is characterized by spontaneous bleeding and pain. To understand mechanisms underlying HA pain, we developed models of *chronic* & *acute* pain in FVIII knock-out (F8KO; B6;129S-F8tm1Kaz/J) male mice using innovative translatable measures to assess pain-related behaviors & gait. We tested for: [i] mechanical hyperalgesia using von-Frey monofilaments; [ii] physical activity using weight-bearing with incapacitance meter and video recordings of free moving mice for locomotion (walking/running), grooming (licking/rubbing body) & rearing (standing); and [iii] gait analysis during normal walking using in-house assembled MouseWalker with high-speed camera & artificial intelligence (AI)-based algorithms.

We observed significant increase in behavioral features of pain starting at ~8wks which continues to 20wks in F8KO Vs age matched B6 ($p<0.05$), including mechanical hyperalgesia, weight-bearing asymmetry, less time in locomotion, rearing & grooming. Gait analysis of ~10wks F8KO showed lowered all-stance, increased body instability, single-swing & posterior extreme position indices to compensate for body instability. This suggests that joint pain may interfere with weight-bearing & locomotion in F8KO, consistent with joint pain, and affect daily activities such as walking in HA patients.

To simulate bleeding-induced " *acute*" pain, we gently inserted 30 G needle into sub patellar position of right hind leg of ~6wks old, anesthetized mice. F8KO showed significantly ($p<0.05$) reduced locomotion, rearing, grooming, %weight applied and increased mechanical sensitivity on injured limb at 4 days post-injury (4D PI) Vs uninjured (UI) F8KO lasting to 4wks PI for most of behavioral parameters suggesting that nociceptive inputs are induced at injury and pain persists after healing.

We observed significant spatiotemporal gait alterations in F8KO PI Vs UI ($p<0.05$ for all features), including reduced stance duration, step length, swing speed and increased swing duration in the injured right hind limb (IRHL) indicative of avoidance of weight bearing on IRHL. To compensate, UI left hind limb (LHL) showed increased stance & reduced swing duration on LHL & left front limb (LFL). These data suggest that F8KO tends to spend shorter ground time on IRHL and longer airtime, compensated by UI LHL & LFL. These observations are complementary to those of mice with sciatic nerve injury & arthritis and patients with rheumatoid arthritis, ankylosing spondylitis, and gout. We observed significant (<0.05) alterations in interlimb coordination with decreased diagonal swing & compensation with increased bound swing, the simultaneous motion of both FLs. A significant increase in all stance index could be compared to increased double support phase & stance phase in patients with HA.

F8KO 4D PI showed significant ($p<0.05$) increase in plasma serum amyloid P & IL6 Vs UI F8KO & injured B6 (IB6). Knee joint at 4D PI Vs UI F8KO & IB6, showed expansion of synovial membrane with increased cellular infiltrate & significant ($p<0.05$) increase in myeloperoxidase, neutrophil elastase +ve cells, degranulating mast cells, calcitonin gene related peptide (CGRP) and substance P (SP). H&E and iron-stained sections of the knee joint ~8wks PI showed significant signs of hemarthropathy ($p<0.05$) Vs UI F8KO & I B6. Interestingly, at ~14wks UI knee joint of F8KO showed significant stromal changes Vs UI B6 demonstrated by increased vasculature, iron stores & mild synovial hyperplasia, indicative of subclinical bleeding that could trigger the transition to chronic pain with age.

The underlying mechanisms involve inflammation, mast cell and neutrophil activation, and vascular changes, perhaps triggered by bleeding. Mast cell tryptase & neutrophil elastase activate protease-activated receptor 2, which may cause nociceptor and endothelial activation, leading to vascular dysfunction and pain. SP and CGRP stimulate vascular permeability and arteriolar dilatation, respectively, and are involved in the generation and maintenance of pain. Thus, neuro-immune and neuro-vascular mechanisms underlie HA pain and may interfere with healing. We show features of pain, gait & weight bear-

ing changes in F8KO mice similar to those of HA patients. Sensory and gait tests used by us have the potential to develop objective measures of pain in HA. Gait changes in F8KO mice precede joint arthropathy, thus may be an early assessment tool in patients with HA.

Disclosures Argueta: *Cyclerion:* Honoraria; *Cayenne Wellness Centers.:* Honoraria.

https://doi.org/10.1182/blood-2023-180380