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Defining the long-term consequences of bone marrow ischaemia.

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Research objectives: Peripheral arterial disease (PAD) affects over 200 million people worldwide and is caused by progressive atherosclerosis limiting lower limb perfusion. Clinical manifestations of PAD include pain, non-healing ulceration and even gangrene, requiring amputation. Perfusion of the bone marrow (BM) is also reduced, but little is known about the consequences of this. We hypothesised that acute disruption of limb perfusion would lead to BM ischaemia, altering its composition and function. We aimed to characterise the kinetics of these perturbations and their potential impact on bone marrow function.

Methods and results: We used a murine unilateral hind limb ischaemia (HLI) model to mimic PAD, with the non-operated limb serving as an internal control, and sham operated mice as further controls. BM composition was characterised using immunofluorescence for endomucin (vasculature), LipidTOX (lipids) and DAPI (nuclei); femur and tibia were imaged at 7- 28- and 61-days after HLI induction. At 7 days post-HLI BM derived macrophages (BMDMs) were isolated, culture for 7 days, then processed for RNA sequencing (RNA-seq) analysis. We histologically confirmed BM ischaemia 7-days post-HLI by detecting pimonidazole adducts; these fully normalised by day 28. Ischaemia at day 7 was associated with a significant increase in vascularity, which normalised by day 61, although there was a sustained BM lipid accumulation from days 7 to 61. A modest decrease in BM leukocyte abundance was observed with flow cytometry on day 61. RNA-seq analysis of BMDMs uncovered 1789 DEGs (adjusted P value <0.05), including 1404 up-regulated and 385 down-regulated genes, in ischaemic limb versus sham operated mice-derived BMDMs. Principal component analysis showed distinct clustering of these groups and pathway analysis highlighted gene-sets related to macrophage motility, amongst others.

Conclusions: Taken together, we demonstrate transient BM microvascular changes and sustained lipid accumulation with reduced leukocyte abundance after inducing BM ischaemia. BMDMs demonstrated markedly altered gene expression even after culture in normoxia for 7 days. Collectively, this could have long term implications for BM function. Further studies are required to define the mechanisms of HLI-induced BM changes, along with the consequences of this.

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