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Chemical characterisation of peripheral signalling in burn injured tissue.

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Aim: Burn pain is excruciating, prevalent world-wide and notoriously difficult to manage (1,2) due in part to the limitations of current therapies. The development of better pain control requires a deeper understanding of nociceptive signalling within the burn injury, as the identities and sensitising/ activating actions on nociceptive afferents of the locally-accumulating biologically active agents are currently poorly characterised. Our aim is to develop methodology for and conduct the definition of the metabolome, lipidome, gene sequencing and cytokine profiles of burn injured tissue.

Methods: Our second degree (3) rat (adult male Sprague-Dawley) burn model consisted of a 60^{o}C scald of one hind paw up to the ankle for 2 minutes under general anaesthesia (I.P. urethane, 1.5 g/kg, 30% in water). Subcutaneous microdialysis was conducted at 2 µl/min to collect half hour fractions of interstitial fluid for 30 mins pre-burn and 3 hours post-burn, both in burned and contralateral (control) paws. Metabolomics utilised Ultra-performance liquid chromatography (Acquity System, Waters); 5 ul of each fraction from four subjects were sequentially injected on a 12 min gradient (4). Mass spectrometry was conducted with a Synapt G2-S Q-ToF (Waters) in positive and negative ionisation modes with acquisition between *m/z* 100 and 1200. 27 cytokines were simultaneously quantified at each time point for four subjects using a multiplex immunoassay (Millipore). Lipidomics and gene sequencing are in progress.

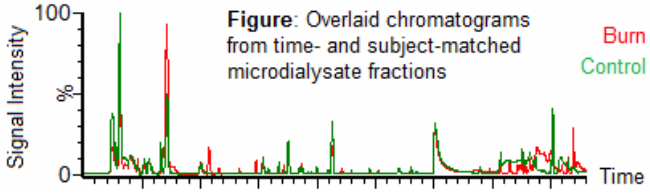

Figure: Overlaid chromatograms from time- and subject-matched microdialysate fractions

Results: Canonical variate analysis (CVA) of the metabolomics data captured stat-istically significant effects for both negative and positive ionisation mode data (P = 1.3 x 10^{-7} and 1.13 x 10^{-7} respectively); metabolites exhibit a robust increase in burn but not control samples over time following the insult. A total of 2808 molecules have been carried forward for further investigation. The cytokine profiling indicated burn-specific up-regulation of two cytokines (P = 6.08 x 10^{-4} and 9.7 = 10^{-5}; 2-way RM ANOVA [Condition (Burn vs. Control) x Time] with Bonferroni correction) not previously implicated in burn injury.

Discussion: The quality of and significant differences observed between burn and control microdialysates support the viability of subcutaneous microdialysis for sampling interstitial fluid. Its suitability for classifying the local milieu in a temporally dynamic system such as during pathological progression is evident. Analyte recovery is sufficient for analysis by mass spectrometry and immunoassays tuned for serum/ plasma, which offer less specific information about local peripheral signalling. In the context of this study, CVA captured the burn-related concentration increases of thousands of compounds; the names and structures of a portion of these will be confirmed and they will be screened for candidate algogens. Along with the

two statistically significant cytokines, their specific implication in burn pain will be investigated *in vitro* in studies designed with the additional incorporation of any information gained from the ongoing lipidomics and gene sequencing components of this study.

Ethical Statement: Experiments were performed in accordance with the requirements of the Animals (Scientific Procedures) Act 1986 (UK) Amendment Regulations 2012 (SI 2012/3039).

References: ***1***. Perry S and Heidrich G (1982). Pain 13: 267-280. ***2***. Carrougher GJ et al. (2003). Journal Burn Care & Rehab 24: 1-8. ***3***. White JP et al. (2011). Eur J Pain 15: 683-690. ***4***. Want EJ et al. (2010). Nat Protoc 5: 1005-1018.