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PhD project title: In vitro analyses of the effects of Vitamin D and allicin in modulation of the inflammatory response as a nutraceutical approach for the aged

University of Registration: University of Leicester

Project outline

1. Project outline describing the scientific rationale of the project

The term “inflammaging” describes a chronic inflammatory condition in the aging population[1]. An increase in prevalence of being overweight or obese contributes to elevated fatty acids[2], circulating endotoxins[3] and inflammatory levels in the aged. This results in added burden on health system resources[4] because chronicity of inflammation significantly contributes to muscle wasting/impaired muscle repair[5], airway hypersensitivity[6] and increased gut epithelial permeability[7].

This project draws on joint cell culture records to investigate interventionist modulation of “inflammaging”. We will use standard hydroxyurea treatment during multiple population doublings to produce replicational stress and thereby mimic aging in culture[8,9] for the cell types of interest: skeletal muscle cells, airway smooth muscle cells, macrophages, and gut epithelial cells.

Using hepatocytes and macrophages, we showed that Vitamin D or docosahexaenoic acid, an omega-3 fatty acid, reduced the inflammatory reactions induced by endotoxin, a pathogen associated molecular pattern (PAMP), while free fatty acids, damage associated molecular patterns (DAMPs), were pro-inflammatory. Our work was published as part of a Frontiers Research Topic “Lipids and the Immune system”[10]. Drs Watson and Smith used exercise as a therapy to reduce age-related loss of muscle mass[11] and quantified a reduction in cytokine expression potentially linked to decreased intramuscular inflammation[12]. Dr Saunders showed that oxidative stress, which is elevated in obesity and contributes to the aging process[13], and the cell stress DAMP HMGB1 increase hypercontractility in airway smooth muscle cells from asthmatics[14,15].

Initially we will characterise the anti-inflammatory response following incubation of target cells with Vitamin D after induction of senescence. This part of the study has the potential to reveal a role for Vitamin D in modulating inflammatory responses in senescent cells by interfering with processes of “inflammaging” and will provide a benchmark via which to assess the efficacy of allicin. Allicin, a key active component of garlic, was proposed as a potential nutraceutical -a dietary component with benefit to health- in obesity associated disease[16].

Recent pilot data demonstrated an anti-inflammatory role of allicin in vitro. The normal TNFα mRNA response elicited by stimulation of macrophages J774 with LPS
*E.coli* 0111B:4 (100ng/ml, 4h) was significantly blunted when cells were preconditioned with 30 μM allicin for one day (80 fold reduction).

Preconditioning of cells with allicin for one day led to a significant reduction of secreted pro-inflammatory cytokine in the supernatant of J774 stimulated with LPS for 24h (unpaired t-test, p<0.05).

We will determine a dose response for allicin and, like for our Vitamin D study, LPS and fatty acids will be used as disease relevant PAMPs and DAMPs to interrogate the potency of allicin to revert the pathological profile to normal.

The mode of action of allicin is likely to be the result of a direct cytoplasmic action with thiol containing proteins[17,18] and will be investigated by proteomic analysis. We will conduct functional analyses of specific cell activities: foam cell production (macrophages), contractility assays (smooth muscle cells), creatine kinase activity (skeletal muscle cells), trans-epithelial permeability assay (gut epithelial cells).

In summary, this project analyses in parallel target cells that are relevant to “inflammaging” in an aged population that is overweight or obese. It investigates the potential of dietary supplements (Vitamin D and allicin) to alleviate inflammatory responses and downstream events that are detrimental to health. On completion, the project will provide comprehensive data with which to formulate a justification for taking forward a possible interventionist approach for this increasing group of society.
References

**Relevant BBSRC Strategic Research Priority:** Bioscience for health

Techniques that will be undertaken during the project.

**Cell culture and stimulation**

ELISA, qPCR, Proteome profile arrays, densitometry, cell signalling via Western blotting

**Microscopy**

Specialist techniques: collagen gel contraction assay to assess smooth muscle contractility, creatine kinase assays for skeletal muscle function, trans-epithelial permeability (TEP) assay

**Statistical evaluations**

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