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Does a 28 Day Exposure To The Fatty Acid Amide Hydrolase Inhibitor, URB597 Prevent Age-Related Dysregulation Of The Lysosomal System And Accompanying Neurodegeneration Within The Male Wistar Rat Brain?

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Endocannabinoids are promising therapeutic agents due to their ability to target multiple mechanisms involved in brain ageing[1]. We have previously shown that endocannabinoids have anti-inflammatory effects in aged rats leading to a restoration of synaptic plasticity[2]. Furthermore, our *in vitro* findings have shown that endocannabinoids can stabilize lysosomes against Aβ-induced permeabilization, prevents the release of lysosomal degradative cathepsin enzymes and inhibits cathepsin-mediated neuronal death[3]. The purpose of this study was to investigate if raising endocannabinoid tone had an affect on the lysosomal system, cell death and inflammasome activity in the cerebral cortex of young and aged male Wistar rats.

Young (3 months; *n*=14, 250–350g) and aged (26-30 months; *n*=14, 550-600g) Wistar rats were randomly divided into those which received subcutaneous (*s.c.*) injections of the FAAH inhibitor URB597 (1mg/kg *s.c.*) every second day and controls which received subcutaneous injections of vehicle (30% DMSO in saline) every second day for 28 days. We have previously shown that this regime elevates endocannabinoid levels in the rat brain[2]. Following the 28-day treatment regime animals were humanely euthanised *via* decapitation, brain tissue was harvested and stored at -80^{o}C before analysis. The integrity of the lysosomal system was assessed by measuring cathepsin activity in cytosolic fractions obtained from cerebral cortical tissue. Cell death in the cerebral cortex was determined by measuring caspase-3 activity. Activities of caspase-1 and cathepsin B were used as markers of inflammasome activation.

Activities of cathepsin D and caspase-3 were significantly increased in cytosolic fractions obtained from cerebral cortical tissue of aged vs young animals (91.6±4.2 RFU/mg protein vs 67.5±7.1 RFU/mg protein for cathepsin D, p=0.035; 2-way ANOVA; 86.7±10.8 RFU/mg protein vs 71.9±14.5 RFU/mg protein for caspase-3, p=0.0029; 2-way ANOVA). Treatment with URB597 reversed the age-related changes in lysosomal instability and cell death as a significant drug treatment affect was observed for cathepsin D ($F_{(1,20)}$ = 11.26, p=0.0032; 2-way ANOVA) and caspase-3 ($F_{(1,22)}$ = 11.26, p=0.0029). Additionally age-related changes in inflammasome activation were also reversed by treatment with URB597 ($F_{(1,10)}$ = 11.10, p=0.0076; 2-way ANOVA, drug affect for cathepsin B; $F_{(1,17)}$ = 7.92, p=0.0120; 2-way ANOVA, drug affect for caspase-1).

Overall, these observations show that a 28-day treatment with URB597 reversed the age-related changes in lysosomal membrane instability, cell death and inflammasome activation.

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1. Gowran A *et al, CNS Neurosci Ther,* 17:637, 2011

2. Murphy N *et al, J Neuroinflammation*, 9:79, 2012

3. Noonan J *et al, J Biol Chem*, 285:38543, 2010