

Project title: Targeted drug delivery to cerebral arteries, using novel peptide-decorated liposomes, for the attenuation of dementia.

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Background: Dementia is a global health problem of increasing prevalence in our ageing population [1]. It is associated with reduced cerebral blood flow and loss of cognitive function [2]. Despite extensive research, there is currently no effective treatment for dementia, partly due to variation in the underlying disease processes. However, a key feature in dementia is deficient neurovascular coupling and impaired dilator function of the cerebral arteries [3], suggesting that these vessels are an important drug target to help improve blood flow to the brain. In ageing and disease, oxidative stress and the generation of reactive oxygen species can quench nitric oxide (a potent vasodilator), leading to endothelial dysfunction and reduced dilator capacity. It is also associated with increased constriction, partly due to nitric oxide's inhibition of potent vasoconstrictors, such as 20-hydroxyeicosatetraenoic acid (20 HETE). The latter is a Cytochrome P450 eicosanoid, which has also been shown to contribute to the development of cerebrovascular diseases [4, 5] and its associated risk factors, such as hypertension [6]. Hence, our aim is to target this system to improve cerebral perfusion and restore vasodilator capacity.

Resveratrol (RV) is a polyphenol with antioxidant properties, shown in clinical trials to improve cerebrovascular function and cognitive performance [7, 8]. However, the evidence remains unclear and there are issues with its oral administration, due to its extensive metabolism in the gut, its low bioavailability and poor stability. One of RV's synthetic analogues, 2,4,3',5'-tetramethoxystilbene (TMS), has more potent actions and has been shown to directly inhibit the CYP450 pathway leading to improved endothelial function [9] and vascular reactivity in spontaneously hypertensive rats [10]. While TMS has improved bioavailability in comparison to RV, it remains at only 4.5% [11]. Hence, we will synthesise a biocompatible drug delivery platform that can target delivery of TMS to small cerebral arteries, hence improve its efficacy.

We will fabricate liposomes and load them with TMS. Liposomes are now recognised as promising brain drug delivery modalities due to their unique characteristics of being biocompatible and capable of manipulation for drug delivery across the blood brain barrier (BBB) [12]. In order to target drug delivery to cerebral arteries, we will tag our liposomes with a peptide sequence that has been demonstrated to cross the BBB and bind to cerebral vasculature [13]. **We hypothesise that targeting TMS to cerebral arteries, using liposomes tagged with homing peptide, will enhance TMS's efficacy and its ability to directly potentiate vasodilation, *ex vivo*.** We will examine the effect of our TMS-loaded liposomes on improving dilation in isolated cerebral arteries taken from normal Wistar rats (after exposure to elevated pressure) and from a transgenic model of Alzheimer's Disease (TgF344-AD), which shows attenuated cerebrovascular reactivity [14]. We will use a bespoke system that maintains small vessels in a viable state to study changes in vessel diameter, in real time. Our future goal is to deliver targeted TMS-loaded liposomes *in vivo* to enhance TMS's bioavailability in endothelial cells and overcome physiological barriers, in particular, the BBB, to restore dilator capacity in dementia.

Work leading to the project: We have previously demonstrated that peptide-decorated, vasodilator loaded liposomes can selectively target the uterine vasculature and improve uteroplacental perfusion in pregnant mice [15]. Using our expertise, we will fabricate liposomes that target the cerebral vasculature. In addition, using isolated endothelial cells, we show that RV-loaded nanostructured lipid carriers induce significant reduction in ROS generation, after hydrogen peroxide exposure. In coronary arteries, we also show that these RV-loaded nanocarriers can restore dilator responses after acute pressure elevation, *ex vivo* [16]. We have the necessary expertise to isolate and mount cerebral arteries (Figure 1) and plan to test dilator effects of the TMS-loaded liposomes.

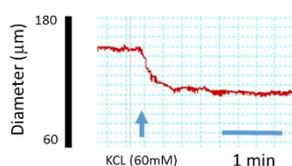


Figure 1: Representative trace showing reduction in cerebral arterial diameter after exposure to high potassium solution, *ex vivo*.

Methodology and work plan:

1. *Synthesise and characterise drug-loaded liposomes.* Lipid nanoparticles (~150 nm) will be prepared using the thin film method as we have previously described [15] and loaded with resveratrol (RV), the RV analogue tetramethoxystilbene (TMS), or the vasodilator SE175, as a positive control. Liposomes will be decorated with the brain homing peptide RLSSVSDLSGC via a Michael-type addition reaction to lipid maleimide groups. This will allow targeting to brain vasculature. We will confirm their size and stability over 1 month and also determine the drug loading efficiency and drug-release characteristics in culture media and physiological salt solution.

2. *Determine effect of drug-loaded liposomes on endothelial cell viability, in vitro.* Primary human endothelial cells will be cultured *in vitro* and the dose response effects of the drug-loaded liposomes on cell viability assessed using our routine alamar blue assay. This will inform appropriate concentrations to use for further study.

3. *Determine the influence of drug-loaded liposomes on the vasodilator response of isolated cerebral arteries, using pressure myography.* We will use our established *ex vivo* model of acute hypertension to increase the level of oxidative stress within cerebral arteries, to represent conditions in ageing and related diseases. These will be isolated from normal Wistar rats and transgenic TgF344-AD rats and maintained under conditions that mimic the physiological state. The influence of drug-loaded liposomes on the endothelial-dependent and independent vasodilator responses will be assessed under normotensive pressure (60 mmHg) and after acute pressure elevation (150 mmHg). We will also assess responses to intraluminal flow. We will relate responses to histological changes in the transgenic brains and also assess liposome uptake into the vessel wall by visualising fluorophores on the homing peptide using fluorescence and confocal microscopy.

References 1. Benjamin et al. *Circulation* 2018; 137: e67- e492. 2. Silvestrini et al.. *Stroke*, 2006;37:1010–1015. 3. Toth et al. *Am J Physiol Heart Circ Physiol.* 2017; 312: H1–H20. 4. Dunn et al. *Am J Physiol: heart Circ Physiol*, 2008; 295:H2455-H2465. 5. Imig et al. *Expert Re Mol Med* 2011; 13: e7. 6. Toth et al. *Am J Physiol: Heart Circ Physiol* 2013; 305:H1698-H1708. 7. Wightman et al, *Br J Nutr*, 2014;112: 203-213. 8. Wong et al, *Nutr Metab Cardiovasc Dis* 2016; 26:393-399. 9. Yuan et al. *Can J Physiol Pharmacol*, 2011; 89:713-722. 10. Jennings et al. *Cardiovasc Drugs Ther* 2014; 28: 145-161. 11. Lin et al. *Biomed Chromatogr* 2010; 24: 1373-1378. 12. Hu et al. *Mol Pharm* 2018; doi: 10.1021/acs.molpharmaceut.8b00611. 13. Urich et al. *Sci Reports* 2015;5 :14104. 14. Joo et al, *Sci Reports*, 2017; 7:46427. **Applicant references:** 15. Cureton N, Korotkova I, Baker B, Greenwood S, Wareing M, Kotamraju VR, Teesalu T, Cellesi F, Tirelli N, Ruoslahti E, Aplin JD, Harris LK. Selective targeting of a novel vasodilator to the uterine vasculature to treat impaired utero placental perfusion in pregnancy. *Theranostics* 2017;7:3715-3731. 16. Astley C, Houcaine C, Singh K, Lightfoot A, Alexander Y, Azzawi M. Resveratrol loaded lipid nanocarriers restore dilator function following acute pressure elevation, *ex vivo*. Under review.

Fit to MICRA call, novelty and expected outcome: Our proposal focuses on developing a novel targeted drug delivery modality for dementia, a growing problem in our aging society, to improve blood flow and cognition. It is a new interdisciplinary collaboration between the University of Manchester: Lynda Harris (expertise in nanocarriers for targeted drug delivery), and Manchester Metropolitan University: May Azzawi (expertise in vascular physiology), Yvonne Alexander (cell and translational biologist), and Llwyd Orton (recently appointed lecturer [<5 yrs], with expertise in neuroscience). The vascular work will be carried out by our PhD student (Cai Astley, MMU), who has acquired skills in vascular function studies, under the PI's supervision. Results will be disseminated at MICRA seminars/events and provide data towards a journal publication to support REF2020. They will form the basis of a grant application to MRC/ARUK/Dunhill Medical Trust. Findings will demonstrate the efficacy of the RV analogue as a treatment option to restore vasodilator function, using peptide-decorated liposomes, enabling development of a novel targeted treatment for dementia patients.

Costings and justification of resources: (1) *Synthesis of the drug loaded liposomes:* cost at @£30/mL. We estimate that we will require 8-10 mL of each liposome type, with and without the surface decorated homing peptide. Cost total including loading drugs (£2,648). (2) *Cell culture:* purchase of primary human cells, media and antibiotics (£550). (3) *Vascular function studies:* Two experimental protocols will be necessary with six vessels each, tested for each liposome type, based on our power calculations. A total of 66 rats will be necessary. Animals will be shared with other projects who will also reciprocate, hence cost requested (£1,200). Cost of the transgenic rats (~£20k) is already covered. Confocal microscopy and histology staining (£1,500). **Grant total cost requested = £5,898.**