Could antiviral & chemotherapy agents have an impact in ME/CFS due to direct effects on mitochondrial function?

Megan Stoker[1], **Daniel Chapero-Hall**[1], Phillip West[2], Karl Morten[1]

NDWRH, The Women's Centre, ***University of Oxford****,* UK[1] *Texas A&M University, Texas,* USA[2]

Introduction

Nucleoside reverse transcriptase inhibitor antivirals have been linked to benefit in some ME/CFS patients. This study looked to test if the antivirals acyclovir (**ACV**) and ganciclovir (**GCV**) and the chemotherapy agent cyclophosphamide (**CP**) had a direct effect on mitochondrial function, a factor suggested to be abnormal in ME/CFS patients. Cellular energetics has been proposed to be impaired in ME/CFS patients with both glycolysis and mitochondrial function being implicated in peripheral blood mononuclear cells (PBMC) [1] and T-cells [2]. It is well established that antiviral therapy using nucleoside reverse transcriptase inhibitors (NRTIs) has off-target effects with a significant effect on mitochondria function in many cases causing mtDNA depletion [3]. This would impact on mitochondrial energy production , mitochondrial morphology and also mitochondrial reactive oxygen species generation all of which would impact on mitochondrial function. In cells dealing with a viral infection (i.e. innate immune cells) this could have a significant effect on how the cells deal with a viral infection. Recent studies by West et al. [4] demonstrate that mitochondrial function and **mtDNA release into the cell plays a critical role in the antiviral response**. We also investigated whether this could be modulated by the presence of a common variant of the mitochondrial DNA (mtDNA), in which there is a single nucleotide polymorphism (SNP) at position 16189. The mtDNA 16189 variant is a known risk factor in the development of type 2 diabetes [5] with evidence that DNA in the triple stranded D-loop from the mutant is held more tightly in the mtDNA molecule (Morten unpublished) which may hinder its release during a viral infection.

Methods

250 µM of ACV, 30 µM of GCV, 10 µM of zalcitabine (ddC) and 100 µM of CP were each tested on various cell lines for 10 days, under **1 mM glucose and 8 % oxygen**. These conditions were chosen to more closely reflect cell physiology in a tissue in vivo. The models used were U87MG human glioblastoma, human rhabdomyosarcoma (RD) and three variants of 143B human osteosarcoma cell lines: wild type (WT), **mtDNA T16189C variant (mutant)** and mtDNA free (ρ0).

T16189C variant

T16189C variant has a single mutation in the D-loop of each mtDNA molecule

In wild type (WT) mitochondrial DNA molecules, the 16189th base is thymine, but the T16189C variant (mutant) contains a cytosine base instead (fig. 1). This single bp difference between wild type and mutant cells is located in the '**D-loop**', a triple stranded segment in the control region of the mtDNA molecule.

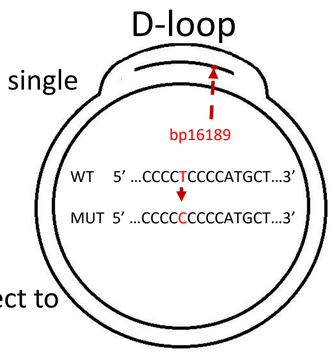

Figure 1. Simple drawing of an mtDNA molecule. The red dot roughly indicates the position of 16189 SNP in the D-loop. The mtDNA of wild type (WT) and mutant (mut.) are also compared.

The T16189C mutation may prevent release of mtDNA into the cytoplasm in times of mitochondrial stress or during a viral infection

Mitochondria provide platforms for antiviral signalling where mtDNA release into the cytoplasm has been shown to trigger the innate immune response and inflammatory pathways through cGAS-STING activation [6]. SNPs in the D-loop may hinder this stress response, increasing the risk of disease, hence we expect to observe cells harbouring the mtDNA 16189C mutant to show inability to give a functional stress response when mitochondria are challenged.

mtDNA Copy Number

Double stranded DNA stain shows no obvious depletion of mtDNA with ACV, GCV or CP

After drug treatment, cells were stained with PicoGreen, which fluoresces upon binding to dsDNA. Bright specks surrounding the nuclei of ACV, GCV & CP treated cells showed that mtDNA was still largely present; ddC left only nuclear DNA visible (fig 2).

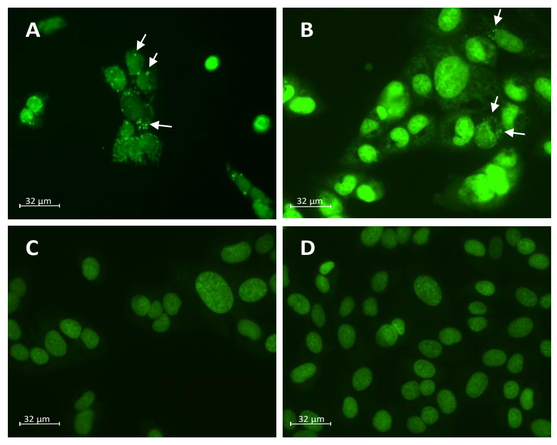

Figure 2. Detection of mtDNA using PicoGreen dye
WT, when untreated (A), GCV-treated (B) and ddC-treated (C), was compared to untreated ρ0 cells (D). Scale bar represents 32 µm. Arrows indicate presence of mtDNA.

qPCR shows change in mtDNA copy number

With ddC treatment, mtDNA:nDNA ratio was reduced to 2.1% of the untreated controls in wild type and mutant 143B cells (p<0.0001) and significantly reduced in U87MG too (fig. 3). ACV showed no significant effect; however, **GCV significantly decreased mtDNA copy number by 58% in wild type 143B cells** (p<0.0001).

Interestingly, **100 µM CP had the opposite effect to the antivirals** in U87MG and mutant 143B cells, with a significant increase in mtDNA copy number. An increase of 161% was observed in U87MG cells (p=0.0112) and an increase of 188% in mutant 143B cells (p<0.0001).

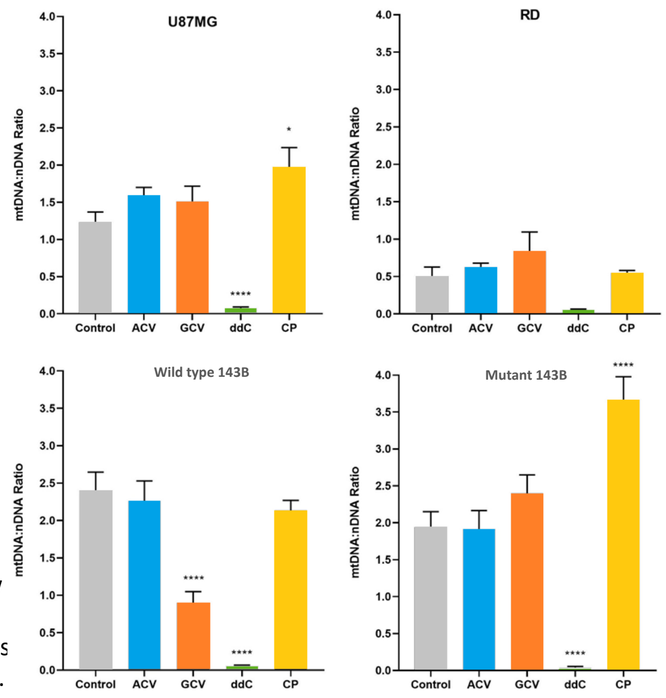

Figure 3. mtDNA:nDNA ratio from qPCR

Bars show mean mtDNA:nDNA ratio; error bars show standard deviation. Significance was calculated by one-way ANOVA using Dunnett Multiple Comparisons test, between treated groups and untreated controls.

Protein Expression

In U87MG cells, ACV increases mitochondrial protein production but CP causes a decrease

In U7MG cells treated with ACV, expression of the mtDNA encoded MTCO1 protein increased (198%, P<0.01) (fig. 4), indicating mitochondrial stress response. However, there was a significant decrease in MTCO1 in CP treated U87MG cells (7%, P<0.01), contrary to the mtDNA upsurge.

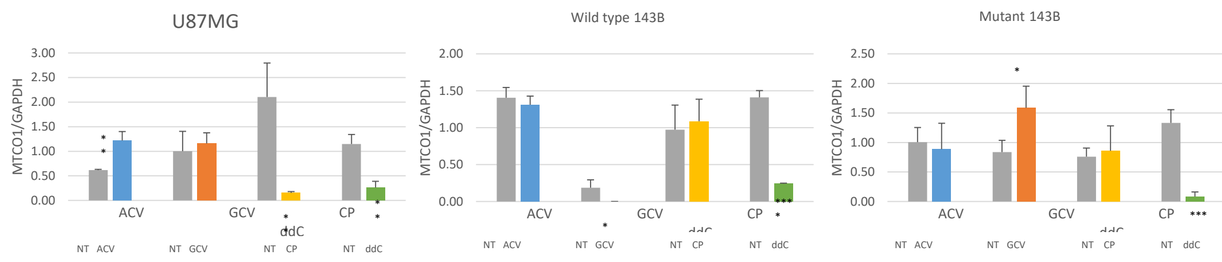

Figure 3. Protein expression following each drug treatment on U87MG and 143B cells
Levels of mtDNA encoded MTCO1 were divided by the levels of the nuclear-encoded protein GAPDH.

The wild type and T16189C mutant exhibit opposite responses to GCV treatment

MTCO1 protein levels completely dropped (0%, P<0.05) in the wild type and significantly increased in mutant 143B (189%, P<0.05) with GCV, showing that the anti-viral impacts mitochondrial function uniquely in two cell lines differing by a single base pair in mtDNA.

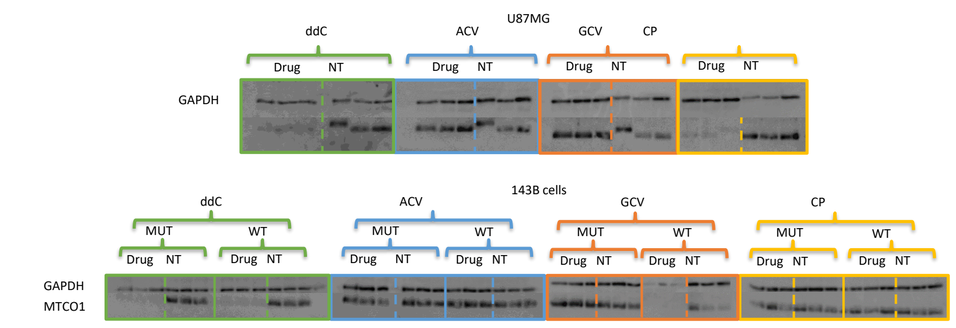

Figure 4. Detection of MTCO1 protein and control protein, GAPDH, following Western Blotting

Conclusions

Following exposure to a range of drugs, distinct patterns of mtDNA and MTCO1 protein expression manifested in each of the cell lines tested, indicating potential variability in the mitochondrial response to treatment within different tissues.

- Mitochondria in the 143B wild type and mtDNA 16189 mutant cells showed contrasting responses to GCV treatment, despite originating from the same cell line, demonstrating that even single nucleotide changes in the D-loop of the mtDNA could significantly alter the mitochondrial stress response. The nuclear genomes of the two lines will likely be identical or very similar as the line was
- Cyclophosphamide treatment also revealed a distinction between the wild type and mutant cells, though only in mtDNA copy number.
- Cyclophosphamide had the most striking effect in U87MG cells, where the levels of the mtDNA-encoded protein MTCO1 were drastically reduced, despite an apparent increase in mtDNA copy number.

The research presented here was carried out as part of the MetaCell-TM project. MetaCell-TM is funded by the EU Horizon 2020 Fast Track to Innovation Pilot (H2020-FTIPilot-2016-1-737978-MetaCell-TM) and the ME Association

References. [1] Tomas, C, *et al*, 2017, Cellular bioenergetics is impaired in patients with chronic fatigue syndrome, PLoS ONE [2] Mandarano, A *et al*, Myalgic encephalomyelitis/chronic fatigue syndrome patients exhibit altered T cell metabolism and cytokine associations, 2019, JCI [3] Wallace, K, Mitochondrial off targets of drug therapy, 2008, Trends in Pharmacological Sciences, Vol 29, p 361-366 [4] West, A, Shadel, G, 2017, Mitochondrial DNA in innate immune responses and inflammatory pathology, Nature Reviews Immunology, Vol 17, p 363–375 [5] Ye, Z *et al*, 2013, The association of the mitochondrial DNA OriB variant with type 2 diabetes in Europid populations, Diabetologia, Vol 56, p 1907–1913, [6] West, A, *et al*, 2015, Mitochondrial DNA stress primes the antiviral innate immune response, Nature, Volume 520, p 553–557