

L-Acetyl Carnitine (LAC) Prevents Antiretroviral Toxic Neuropathy (ATN) in an *In Vitro* Model.

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BACKGROUND

Sensory neuropathy is the commonest neurological complication of HIV infection, and affects 44% of patients attending the Alfred Hospital HIV Clinic.¹ Most cases in this clinic are due to antiretroviral toxic neuropathy (ATN) with the main risk factor being exposure to d4T or ddl: both potentially neurotoxic nucleoside analogs (NRTIs). An open-label study in 23 patients has suggested LAC in a dose of 1500mg twice daily may improve ATN,² but data to support the biological plausibility of this approach are lacking.

Both the antiviral effects and the toxicity of NRTIs are thought to occur through similar mechanisms, with these medications also acting as substrates for cellular DNA polymerases.³ It is therefore critical to ensure that any substance that prevents the toxicity of NRTIs does not also reduce their efficacy.

AIMS

1. To confirm cultured fetal rat dorsal root ganglia (DRGs) as an *in vitro* model of ATN.⁴
2. To use this model to examine the efficacy of LAC in preventing NRTI neurotoxicity
3. To examine the effect of LAC on the antiviral efficacy of NRTIs *in vitro*.

METHODS

Fetal rat DRGs were obtained from E15 Sprague Dawley rats and cultured on collagen coated plates with nerve growth factor. NRTIs (NIH AIDS Reagent Program) and/or LAC (Sigma-Aldrich) were added to the collagen in concentrations based on estimated serum levels in patients exposed to therapeutic doses of each agent (therapeutic) or three fold higher (high dose) (Table 1). At least 16 DRGs were cultured with each dose of drug. DRGs were monitored over time in terms of histology and length of neurite outgrowth using video image analysis.

Drug	Therapeutic	High Dose
Zidovudine (AZT)	7µM	21µM
Lamivudine (3TC)	8.3µM	24.9µM
Abacavir (ABA)	10.4µM	31.2µM
Didanosine (ddl)	11µM	33µM
Stavudine (d4T)	3.6µM	10.8µM
Zalcitabine(ddC)	177nM	531nM
L-acetyl carnitine (LAC)	50µM	-

The effect of LAC on the antiviral efficacy of NRTIs was assessed in MT-2 cells. Cells were infected with a standard inoculum of HIV in the presence of a range of concentrations of AZT with and without LAC. The One Solution Cell Proliferation Assay (Promega) which is an MTT based assay was used to estimate cell viability.⁵

RESULTS

DRGs as a model of ATN

Fetal rat DRGs appear to be a robust model for ATN.

Dose dependent toxicity was seen with all of ddl, d4T and ddC, the three NRTIs that have been associated with ATN in clinical use (Figure 1) No toxicity was observed when DRGs were cultured in the presence of AZT, 3TC or abacavir, NRTIs that have not been associated with ATN clinically.

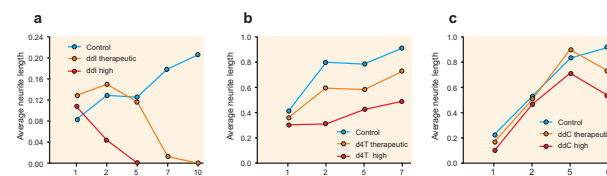


Figure 1: Dose-dependent toxicity from ddl (a), d4T (b) and ddC (c) seen in reduced DRG neurite growth compared with controls.

The effect of LAC on NRTI toxicity in DRGs

The co-administration of 50µM LAC effectively prevented the toxicity of even high doses of ddl, d4T and ddC in fetal rat DRGs.

The effect of LAC on ddl toxicity in DRGs

DRGs cultured with high dose ddl demonstrated reduced neurite growth relative to controls by day 2 ($p=0.02$, unpaired t test), and were all dead by day 5. DRGs cultured with high dose ddl and LAC remained healthy to at least day 10 and had neurite outgrowth similar to controls ($p=0.9$) (Figure 2). Further, histology of DRGs grown with high dose ddl and LAC remained similar to that of controls out to time points where DRGs grown with ddl alone were dead (Figure 3).

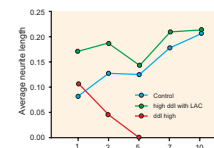


Figure 2: Neurite growth over time in DRGs cultured in the presence of high dose ddl +/- LAC compared with controls

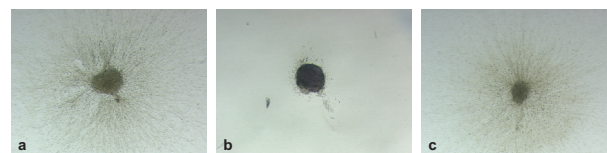


Figure 3: Histology of DRGs at day 5 of culture. Control DRGs (a) and DRGs grown with high dose ddl and LAC (c) look healthy, whereas DRGs grown with the same dose of ddl alone (b) are dead.

RESULTS

The effect of LAC on d4T toxicity in DRGs

DRGs cultured with high dose d4T showed significant impairment of neurite growth by day 2 (60% reduced neurite length versus controls, $p<0.0001$). DRGs cultured with high dose d4T plus LAC demonstrated similar neurite growth to controls to at least day 7 ($p=0.2$) (Figure 4).

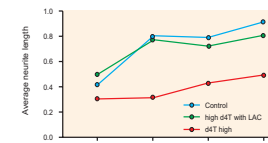


Figure 4: Neurite growth over time in DRGs cultured in the presence of high dose d4T +/- LAC compared with controls

The effect of LAC on ddC toxicity in DRGs

Fetal rat DRGs grown in the presence of ddC exhibited less profound inhibition of neurite outgrowth than those grown in the presence of ddl or d4T, perhaps relating to species differences in the intracellular activation of NRTIs into their tri-phosphorylated forms. Nonetheless, the co-administration of LAC did offset the toxicity of high dose ddC in this model (Figure 5).

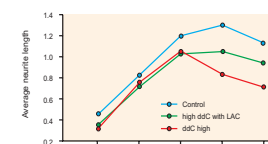


Figure 5: Neurite growth over time in DRGs cultured in the presence of high dose ddC +/- LAC compared with controls

The effect of LAC on NRTI antiviral efficacy

The addition of 50µM LAC did not increase the IC_{50} of AZT in MT-2 cells (IC_{50} AZT = 0.0084µM, IC_{50} AZT+LAC = 0.006µM) Therefore we saw no evidence that LAC impairs the antiviral efficacy of AZT under the conditions of this assay (figure 6).

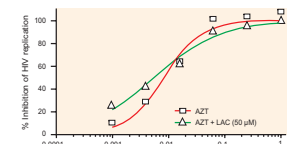


Figure 6: The addition of 50µM LAC does not significantly alter the IC_{50} of AZT in MT-2 cells

CONCLUSIONS

1. Fetal rat DRGs are a useful model of ATN, with toxicity seen only from those NRTIs associated with ATN clinically.
2. LAC prevents ddl, d4T and ddC toxicity in this model, suggesting a role for LAC in preventing ATN.
3. LAC does not impair the antiviral efficacy of AZT in the MT-2 cell model used.
4. Controlled trials will be needed to confirm the efficacy of LAC in a clinical setting, and to determine whether it has a role in the treatment of established ATN.

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