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Towards an Understanding of the Side Effects of Anti-HIV Drugs Using Caenorhabditis Elegans

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Summary/Samenvatting

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To date, human immunodeficiency virus (HIV) infection is one of the most important global health issues, with approximately 35 million people infected worldwide and 2.1 million newly infected each year. HIV-1 infects cells of the immune system and uses them to replicate, leading to their demise. After a while the immune system of the patient becomes compromised to the extent that opportunistic pathogens can easily infect the host. If HIV-1 infection is left untreated it can lead to immune-incompetence and acquired immune deficiency syndrome (AIDS), which quickly leads to death.

Since the discovery of HIV-1 as a cause for AIDS, many antiretroviral drugs have been developed to target viral replication. There are currently six different classes of anti-HIV drugs which each act on a particular aspect of the viral life cycle. The first class of antiretroviral drugs to be utilized in the clinic was the Nucleoside Reverse Transcriptase Inhibitors (NRTIs). These drugs inhibit the conversion of viral RNA into viral DNA, a critical step in the HIV-1 replication cycle. Protease Inhibitors (PIs) were the second class of antiretroviral drugs to be administered in the clinic for HIV-1 infection. PIs hinder the cleavage of immature viral proteins into mature viral proteins and in this way inhibit the production of new infectious virus particles. NRTIs and PIs are used in unison to increase therapy efficacy, overcome problems of tolerance, and decrease the emergence of viral resistance. The therapeutic use of a combination of drugs, more commonly known as Highly Active Anti-Retroviral Therapy (HAART), was a major advance in HIV therapy and has significantly improved the quality and length of patient lives.

Overshadowing this celebrated success, however, is the problem that HIV-1 infected patients are afflicted with drug induced adverse events, some of which can be life threatening. For instance, patients treated with HAART can suffer from metabolic diseases and organ failure, and can show signs of premature ageing. As antiretroviral drugs do not cure HIV-1 infection, but only decrease viral replication, patients have to take HAART for the rest of their lives, therefore increasing the chance of adverse events. Most adverse events witnessed by patients using antiretroviral therapy seem to be related to tissues with high-energy demand and have predominantly been found to be caused by mitochondrial toxicity. Mitochondria are often called the ‘power-houses’ of the cell as they generate energy that is vital for life. Disruption of mitochondrial function can therefore cause many adverse events and even lead to death.

NRTIs have been shown to have relatively high affinity for the mitochondrial polymerase-γ, the enzyme responsible for mitochondrial DNA (mtDNA) replication and repair. MtDNA encodes proteins that constitute vital parts of the mitochondrial respiratory chain (MRC), the cellular machinery necessary to biochemically produce energy in the form of adenosine triphosphate (ATP). By inhibiting polymerase-γ, NRTIs disrupt the production of energy and in this way cause mitochondrial dysfunction; a process commonly referred to as the polymerase-γ theory.

A direct mode of action behind PI induced mitochondrial toxicity remains to be elucidated, although theories have been formulated. These revolve around the increased generation of mitochondrially derived reactive oxygen species (ROS). ROS are potentially harmful products of oxidative phosphorylation that takes place in the mitochondria, specifically at the site of the MRC. ROS can damage proteins, lipids and nucleic acids and in this way have been proposed to cause adverse events and accelerate the ageing process.

In **chapter 1** the side-effects of NRTIs and PIs are summarised. Current and novel theories underpinning their adverse events and mitochondrial toxicity as common denominator in these models are discussed. I also recapitulate our proposed theory that mitochondrial toxicity is likely an underlying cause for the premature and accelerated ageing observed in HIV-1 treated patients. This chapter ends with an overview of major questions that remain to be addressed in this research field.

Having set the playing field, I verify in **chapter 2** that *C. elegans* can be used as a model system to study the adverse side effects of HIV-1 antiretroviral medicines. Using an array of established and novel molecular techniques I show that although NRTIs are supposed to have similar modes of action causing toxicity, they each have distinct toxicity profiles. Additionally, I show evidence in support of an earlier proposition, namely that there are modes to NRTI toxicity beyond the polymerase-γ theory.

In **chapter 3** my hypothesis that NRTIs cause premature and accelerated aging, as proposed in chapter 1, is assessed. I show that NRTIs can directly inhibit the MRC without relying on mtDNA depletion, which is in contradiction to the polymerase-γ theory. MRC inhibition induces an immediate ROS stress response and a reduction in mitochondrial function that likely leads to mitohormetic events; an increase in stress resistance, prolonged lifespan but reduced fitness, which is similar to phenotypes portrayed by *C. elegans* mitochondrial (*mit*) mutants. Additionally, I show that mitochondrial dysfunction and the mitohormetic phenotypes observed can be attenuated by antioxidants.

The polymerase-γ theory is currently the major theory explaining mitochondrial toxicity and adverse events of NRTIs. Recently this theory has been questioned as data has accumulated over the years that suggest that there are other modes of NRTI toxicity. In **chapter 4**, genome-wide transcriptome analysis is utilized to unbiasedly research NRTI thymidine analogue effects on mitochondria and the cell as a whole. I show that some NRTI thymidine analogues disturb energy metabolism without depleting mtDNA. In support of one theory of NRTI toxicity beyond polymerase-γ inhibition, I propose that thymidine analogues disrupt the homeostasis of endogenous nucleoside pools, which is especially of consequence during mitosis. Additionally, I demonstrate that thymidine analogue induced gene expression is similar to gene expression profiles in *mit* mutants and in nematodes treated with the pro-oxidant paraquat, supporting our findings in chapter 3.

Mitochondrial toxicity of PIs has been less well researched than NRTIs. In **chapter 5** I focus on PIs and their effect on *C. elegans* and specifically their ability to disrupt mitochondrial function. I show that PIs cause rapid mitochondrial dysfunction and initiate ROS stress responses, which are accompanied by a severe reduction in

fitness. Interestingly, many of these side effects could be attenuated by antioxidants, supporting the theory that ROS generation is pivotal in PI induced mitochondrial toxicity.

Many antiretroviral medicines are used in combination for HIV-1 therapy. In **chapter 6** I screen combinations of NRTIs and PIs for their ability to induce mitochondrial toxicity. This toxicity profiling is then correlated to clinical data on combination therapies in an attempt to verify and create new knowledge on which combinations are particularly toxic or those which show fewer side effects.

In **chapter 7** a brief summary of the previous chapters is given together with a critical comprehensive discussion of the results and conclusions of this thesis. Additionally, chapter 7 focuses on the use of *Caenorhabditis elegans* as a model system to study the effects of drugs, in particular on the mitochondria. A short comparison of *C. elegans* and human mitochondria is given along with practical challenges that the use of *C. elegans* entails.