Clinical evaluation of systems pharmacology interventions for NAD-enhancement

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Introduction

Systems pharmacology & ageing

Despite the observation that seemingly simple interventions such as a single gene mutation can have major effects on lifespan, ageing is intrinsically complex, being driven by multiple causal mechanisms (Kirkwood et al. 2011). These driving mechanisms have been categorized into nine hallmarks of ageing (Lopez-Otin et al. 2013), but in reality the molecular and cellular mechanisms underlying each hallmark themselves are multiple and complex. The reason for this complexity is that evolution has selected biological function for robustness (Masel and Trotter 2010). Raman and Wiltshire (2012) have identified the systematic biology of the human cell as composed of complex sets of pathways, feedback loops, signalling sequences, and competitive interactions (Young 1992, 1993; Young et al. 2000; Zimmer and Young 2009). This means that complex biological processes such as ageing cannot be understood through the application of a single target reductionist approach, strengthening the argument for a multi-targeted approach. Whilst multiple targeted interventions in a biological network have been shown to produce greater network impact in some cases, a caveat is that combinations of simultaneous interventions that yield very high network impact are unlikely to be found unless they are specifically optimised or searched for in a scientifically rational way (Zimmer and Young 2009). Systems pharmacology addresses this complexity by, for example, using network impact analysis to specifically target network properties rather than the properties of single components, such as single proteins, to establish methods and tools that can be used to test these high impact combinations.

NAD & ageing

NAD and its metabolism have become a major focus for research into ageing for a number of reasons. As well as its central role in energy metabolism, NAD appears to be a component of important signalling mechanisms, through which it affects the investment that cells make in maintenance and repair processes in general and DNA repair in particular (e.g. Croteau et al. 2017). NAD levels decline exponentially throughout life, mirroring the decline in related biomarkers (Gomes et al. 2013; Inal & Guerente 2016) and the causal intervention of resetting the intracellular levels of NAD in mammals fully reverts to a young state at younger age in a variety of age-related markers to those consistent with a younger state (Zhao et al. 2019). These results suggest that NAD is a highly compatible form to mitigate some of the effects of intrinsic ageing in people. These “therapeutic” approaches have so far centred around dietary supplementation of NAD precursors, typically NMN and nicotinamide riboside (NR), and direct infusion of NAD (Trammell et al. 2016; Mills et al. 2016; Martens et al. 2018). Here we describe the use of a systems pharmacology approach to identify interventions that deliver substantial and sustained enhancements in NAD levels for greater healthspan methods.

Results

Circadian variability

Cellular NAD levels, NAMPT expression and SIRT1 activity are all known to exhibit significant circadian oscillation, with NAD reportedly fluctuating by 2.5-fold throughout the day (Nakahata et al. 2009). Therefore, to ensure that any observed variation in participant NAD levels were due to the intervention and not simply natural daily fluctuations, we closely measured and matched participants baseline NAD levels prior to the intervention (Figure 1). Blood samples were taken every 2 hours for 3 days and NAD measured to determine individual NAD patterns and NAD measurements throughout the intervention period were then performed at each participant’s lowest daily NAD point. Figure 1 shows the average NAD levels measured in FBMCs of one of our participants across a 12-hour time frame.

Intervention NCDC01.1

NCDC01.1 is a combination of a small number of molecules, designed using a systems pharmacology approach to upregulate NAD anabolic enzymes, such as those that generate NAD from its precursors and other metabolites, and to downregulate NAD catabolic enzymes, alongside an NAD precursor. During the intervention phase, participants' blood was harvested every other day and NAD levels in FBMCs measured. Across a 14-16 day exposure, NAD titres rose on average 344% of baseline levels (Figure 2A and 2B).

Intervention NCDC02.0

NCDC02.0 is a combination of a small number of molecules that do not include an NAD precursor. Alongside molecules intended to upregulate activity in NAD anabolic pathways, it included molecules designed to downregulate NAD catabolic pathways in favour of NAD salvage pathway recycling. During a seven-day exposure in a human volunteer, NAD titres rose 170% of baseline NAD levels in FBMCs (Figure 3). This is comparable with NAD enhancements achieved with precursor supplementation (Trammell et al. 2016). NCDC02.0 was thus designed to represent a fairly full expression of a systems pharmacology approach to increasing and sustaining NAD titres in man. With this intervention we achieved an order of magnitude higher NAD enhancement than for NR-based precursor supplements (e.g. Trammell et al. 2016). As far as we know, this is the largest sustained increase in NAD titres so far reported in man.

There is growing evidence to suggest that plasma levels of most NAD precursors are probably unable to systematically sustain high NAD production rates (Canto et al. 2016). Consequently, it seems that mammalian organisms largely rely on NAD’ salvage from intracellular NAM in order to maintain NAD pools (Hoitink et al. 2018). Our results show that these substantial levels may be at least in part due to increased expression of the NAMPT enzyme which is thought to be the rate-limiting step in NAD salvage pathway.

Finally, analysis of downstream changes in age-related biomarkers revealed that SIRT1, an NAD-dependent deacetylase that is believed to mediate many of the metabolic responses to calorie restriction and to play a role in positive regulation of DNA repair was substantially upregulated in response to exposure to NCDC02, suggesting that some of the hoped-for downstream benefits of restored NAD titres may be becoming evident.

Our data demonstrate that interventions to enhance NAD are not limited to precursor supplementation, and that far greater enhancement in NAD levels can be achieved with a multi-target approach designed to act upon the enzymatic networks that build and break down NAD within mammalian cells.

Discussion

To date, therapeutic approaches to enhance NAD levels that decline with age have centred around dietary supplementation of NAD precursors, typically NAD, nicotinamide riboside (NR), and direct infusion of NAD (Trammell et al. 2016; Mills et al. 2016; Martens et al. 2018). We were interested in the fact that NAD is one metabolite of a complex set of multifunctionalenzymes. If the properties of these networks are not themselves beneficially altered, then supplementation with NAD precursors ultimately results in a ceiling effect: an increase of NAD titres corresponding to the level at which NAD catabolic pathways are upregulated (Kraus et al. 2014; Trammell et al. 2016). Accordingly, we explored a multi-system level approach to develop a substantial and sustained increase in NAD levels. We designed a number of combination interventions with these aims, and derived clinical data from analysis of blood samples from human subjects.

In summary, NCDC01.1 initially proved that a systems approach can uplift NAD more than precursor supplementation alone. To further validate our approach, we then designed NCDC02.0 which did not contain any NAD precursor. This intervention increased NAD levels comparably to NAD enhancements achieved with precursor supplementation (Trammell et al. 2016). NCDC02.0 was thus designed to represent a fairly full expression of a systems pharmacology approach to increasing and sustaining NAD titres in man. With this intervention we achieved an order of magnitude higher NAD enhancement than for NR-based precursor supplements (eg. Trammell et al. 2016). As far as we know, this is the largest sustained increase in NAD titres so far reported in man.

Methods

Systems pharmacology

NCDC01.1 is the combination of a small number of molecules that can be used to exhibit substantial and sustained enhancement in NAD levels for greater healthspan and disease resistance.

Intervention NCDC01.1

NCDC01.1 is a combination of a small number of molecules, designed using a systems pharmacology approach to upregulate NAD anabolic enzymes, such as those that generate NAD from its precursors and other metabolites, and to downregulate NAD catabolic enzymes, alongside an NAD precursor. During the intervention phase, participants’ blood was harvested every other day and NAD levels in FBMCs measured. Across a 14-16 day exposure, NAD titres rose on average 344% of baseline levels (Figure 2A and 2B).

NAMPT expression

In mammals, nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme in the NAD salvage pathway (NAD+ salvage), and is involved in the expression of NAMPT using Western blot analysis (Figure 5). NAMPT expression was found to increase over a 16-day exposure to NCDC01.1.

SIRT1 expression

Many of the beneficial effects of elevated NAD levels are believed to be due to its role as a co-substrate for the sirtuins. Therefore, participants’ FBMCs were also analysed for changes in the expression of the sirtuins using Western blot analysis (Figure 5). SIRT1 expression was also found to increase across a 16-day exposure to NCDC01.1.

References