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Sirtuins: Novel targets for metabolic disease

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Sirtuins represent a novel family of enzymes that are collectively well situated to help regulate nutrient sensing and utilization, metabolic rate and ultimately metabolic disease. Activation of one of these enzymes, SIRT1, leads to enhanced activity of multiple proteins, including peroxisome-proliferator activated receptor coactivator-1α (PGC-1α), which helps to mediate some of the in vitro and in vivo effects of sirtuins. As such, enhanced SIRT1 activity decreases glucose levels, improves insulin sensitivity, increases mitochondrial number and function, decreases adiposity, improves exercise tolerance and potentially lowers body weight. SRT-501 is a proprietary formulation of resveratrol with improved bioavailability. As such, SRT-501 represents the first in a novel class of SIRT1 activators that has proven to be safe and well-tolerated in humans. Clinical trials in type 2 diabetic patients are currently underway.

Keywords Calorie restriction, diabetes, mitochondria, PGC-1α, resveratrol, SIRT1, SRT501

Introduction

The identification of mammalian sirtuins (SIRTs; silent information regulator transcript) with close homology to Sir2 from yeast is a recent discovery, which has ignited the field of caloric restriction (CR) and metabolic control [1,2]. CR is one of the best known methods for extending a healthy lifespan. The increased longevity associated with CR results from reduced rates of cancer, diabetes, inflammation and cardiovascular diseases. The quest to understand how these events are linked has been spearheaded by the seminal research of Sinclair and Guarente, who demonstrated that activation of the sirtuin, SIRT1, appears to be critical for the effects of CR in yeast and more complex eukaryotes, including mammals [3-8]. Some of these metabolic findings appear to have been confirmed in human volunteers undergoing CR, who have displayed similar improvements to those seen in animals [9••].

The seven mammalian sirtuins identified appear to have differential cellular locations and as such, potentially different physiological roles (Figure 1) [10,11]. Furthermore, it appears that specific sirtuins may offer function in a tissue- and subcellular-specific manner leading to enhanced specificity of those molecules designed to modulate their activity [12].

The sirtuins are classified together according to their sequence similarity and inherent requirement for nicotinamide adenine dinucleotide (NAD) to elicit their associated functions. Although the sirtuins are generally thought of as deacetylases, at least two family members, SIRT4 and SIRT6, exhibit ADP-ribosyltransferase activity [13]. The gene location of each sirtuin and their downstream protein targets provide multiple opportunities to interface with mechanisms that control metabolic state. Furthermore, their uniqueness provides opportunities for their modulation and the subsequent development of selective agents designed for specific therapeutic targets [11].

A vast majority of publications on sirtuins and metabolic disease have focused on SIRT1. This trend is driven by the association of SIRT1 activity with CR and the availability of a SIRT1 activator, resveratrol (RM-1812, SRT-501, Royalmount Pharma/Sirtris Pharmaceuticals Inc, Figure 2).

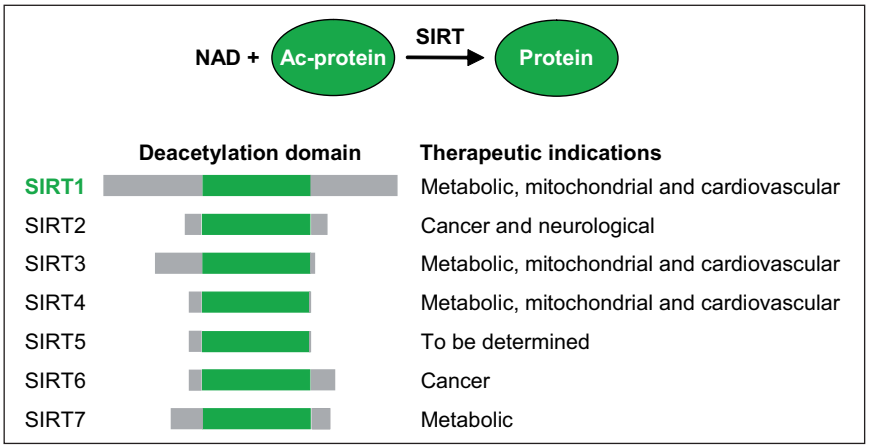
Resveratrol, a component in many red wines, has been demonstrated to activate SIRT1 and extends lifespan in multiple model organisms [14,15]. In mice receiving a high-calorie diet, resveratrol has also displayed positive effects on outcomes in multiple disease states including cancer, heart disease, inflammation, cardiovascular diseases, ageing and metabolic diseases [16••].

While the most prolifically published data surrounds SIRT1 with regard to metabolic regulation, other sirtuins are clearly getting in on the act. For example, SIRT4 has been implicated in the modulation of insulin secretion [17,18••,19] and SIRT3 may also become an attractive target for metabolic diseases [20•]. This review highlights some of the milestone developments that have helped forge the novel and exciting field of sirtuin biology.

Molecular basis of SIRT1 modulation

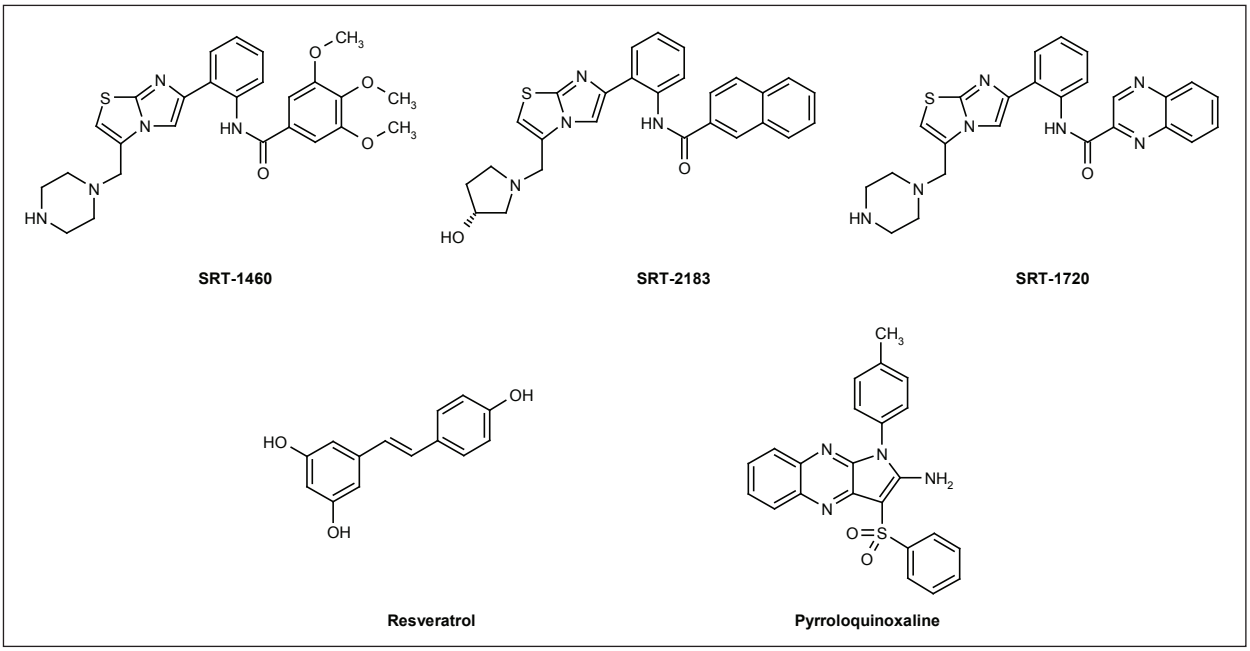
SIRT1 is the most well studied of the seven sirtuin family members and functions as a protein deacetylase that requires NAD as a cofactor. The human *SIRT1* gene (Figure 3A and 3B) has been traced to chromosome 10q21.3 and spans a region of 33,660 base pairs (bp). Preceding the open reading frame is a CCAAT-box, several NFκB

Figure 1. Sirtuin family of enzymes.



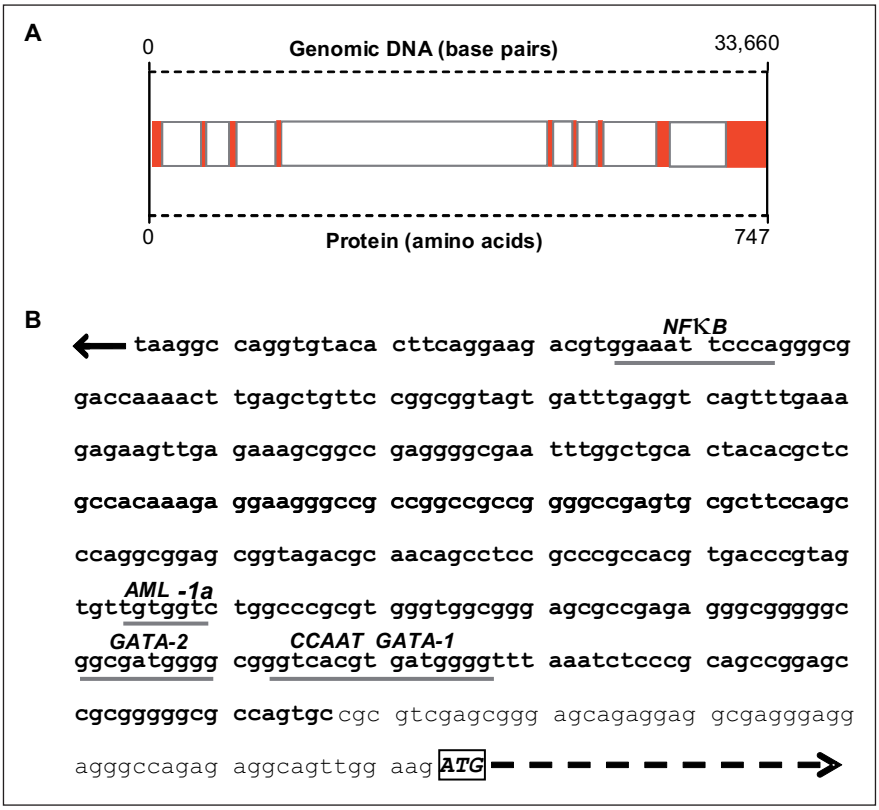
A schematic diagram displaying how each sirtuin relies on the presence of NAD to modify target proteins. The structural similarity of the seven known mammalian sirtuins and their common active site are shown. **Ac-protein** acetylated protein.

Figure 2. The structures of SIRT1 activators.



and GATA-1 and -2 transcription factor binding sites and a 350-bp CpG island. SIRT1 is encoded by 9 exons and the 4107-bp human SIRT1 mRNA has an open reading frame of 2244 bp and encodes a 747-amino-acid protein [21]. SIRT1 mRNA binds to the human RNA-binding protein HuR and is released upon oxidative stress induced by hydrogen peroxide [22]. Transcription of *SIRT1* is induced by E2F transcription factor 1 (E2F1) and, in turn, SIRT1 inhibits E2F1 activity, producing a negative feedback loop [23]. *SIRT1* transcription can also be modulated by nutrient deprivation and can be mediated by HIC1 (hypermethylated in cancer 1) through a decrease of C-terminal-binding protein (CtBP) binding and subsequent increase in SIRT1 expression [24,25]. Furthermore, SIRT1 levels are increased upon CR in humans [26]. The enzymatic deacetylase function of the Sir2 family has been studied in detail and an overall reaction is depicted in Figure 4. The use of NAD as a cofactor produces a second messenger 2′/3′-*O*-acetyl-ADP-ribose (2′/3′-O-AADPR) in addition to ε-aminolysyl residues in the protein substrate [6,27]. Some of the substrates for SIRT1 deacetylation include: retinoblastoma protein [28], the tumor suppressor HIC1 [29], p53 [30], Ku70 [31], NFκB [32,33], the *Drosophila* forkhead-related (FoxO) transcription factors FoxO1 [34], FoxO3a, FoxO4 and FoxO6 [35], and peroxisome-proliferator activated receptor coactivator-1α (PGC-1α) [36,37••]. The integrated action of SIRT1 on these and other co-regulators functioning as activators or repressors produces an improved metabolic profile upon SIRT1 activation in the face of a metabolic stress such as type 2 diabetes (Figure 5) [38]. Whole-body overexpression of SIRT1 in mice produces

Figure 3. Details of the *SIRT1* gene.



(**A**) Schematic diagram of the exon and intron organization of *SIRT1* to scale. The *SIRT1* gene encodes 9 exons (gray) separated by 8 introns (white). The genomic region of the *SIRT1* gene covers 33,660 base pairs and encodes a protein of 747 amino acids. (**B**) Schematic diagram of the *SIRT1* gene showing promoter elements and intron and exon structure. The 5' upstream promoter region of the *SIRT1* gene contains NFκB, AML-1a, GATA-2, CCAAT, and GATA-1 transcription factor binding sites (underscored) and a small CpG island (bold).

improved insulin sensitivity and glucose homeostasis [39]. SIRT1 overexpression in liver is an important factor in the regulation of glucose and lipid homeostasis in the fasting state [40]. In the insulin-resistant and diabetic state, SIRT1 activation improves whole-body insulin sensitivity and glucose homeostasis and improves hepatic, muscle and adipose insulin sensitivity while reducing inappropriate hepatic gluconeogenesis [41••]. In addition to improved insulin sensitivity and glucose homeostasis, activation of SIRT1 has positive effects on insulin secretion and biosynthesis as well as the potential to improve β-cell function through suppression of mitochondrial uncoupling protein 2 (UCP2) [42].

Small-molecule SIRT1 modulators

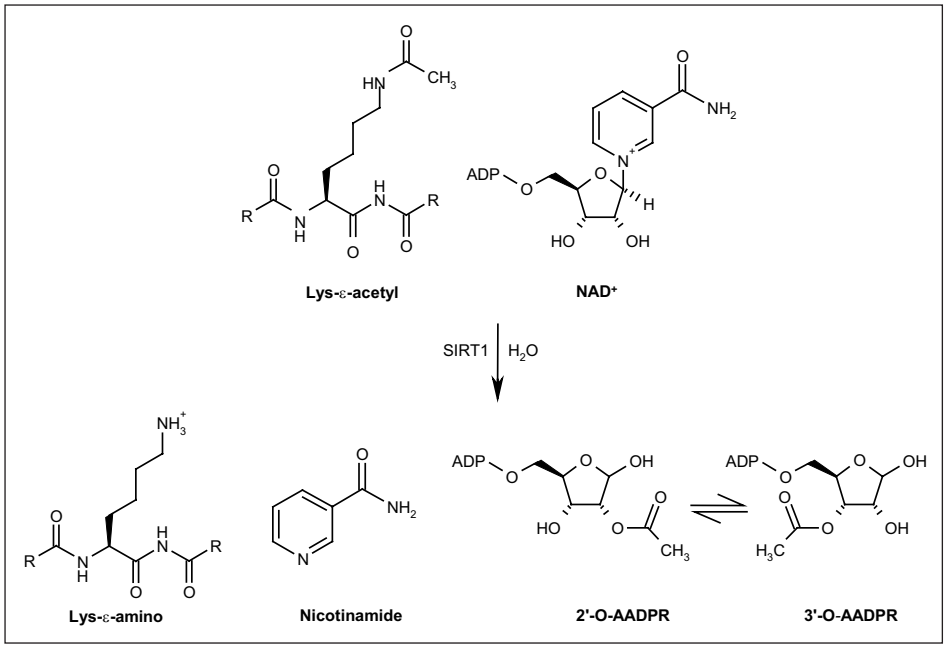
SIRT1 inhibitors

Several small-molecule SIRT1 inhibitors beyond nicotinamide have been reported in the literature and the structures of these compounds are shown in Figure 6. Sirtinol has been used extensively in the literature as a SIRT1 inhibitor. Other inhibitors have been derived from extensive structure-activity relationship (SAR) studies utilizing an indole starting point and producing tetrahydrocarbazoles (for example, the middle compound of Figure 6) as well as starting from splitomycin to produce HR-73 (Figure 6) [43-45].

SIRT1 activators

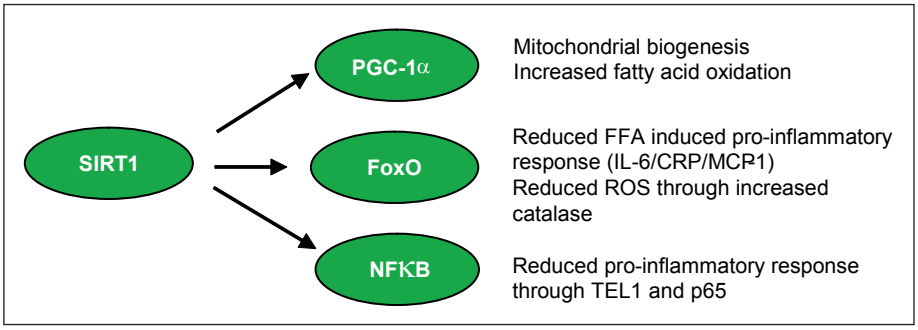
The utilization of SIRT1 activators has been recognized as an important new potential therapeutic approach to treat multiple disease states including neurodegenerative and metabolic disease in addition to cancer [46]. Resveratrol (Figure 2) is a SIRT1 activator that has been well described in the literature, and causes approximately 2-fold activation (201% maximal activation) of SIRT1 with an $EC_{1.5}$ value of 46 μM (the $EC_{1.5}$ value is the concentration of compound required to increase SIRT1 activation by 50%), as determined utilizing a mass spectrometry assay [41••]. The SAR of resveratrol has been explored for SIRT1 activation, and substituents at the 3 and 5 position of the A ring (see Figure 2) were identified as being critical for activity, while substituents at the 4' position of the B ring were less sensitive to substitution [47]. Recently a series of compounds that are structurally unrelated to resveratrol and are 1000-fold more potent has been described (Figure 2) [41••]. Interestingly, these compounds, SRT-1720 ($EC_{1.5}$ = 0.16 μM, 781% maximal SIRT1 activation), SRT-1460 ($EC_{1.5}$ = 2.9 μM, 446% maximal SIRT1 activation) and SRT-2183 ($EC_{1.5}$ = 0.36 μM, 296% maximal SIRT1 activation), are able to induce 3- to 7-fold maximal activation of SIRT1 and the percentage of SIRT1 maximal activation may provide unique pharmacological profiles for these agents in addressing different disease indications.

Figure 4. The reaction of SIRT1.



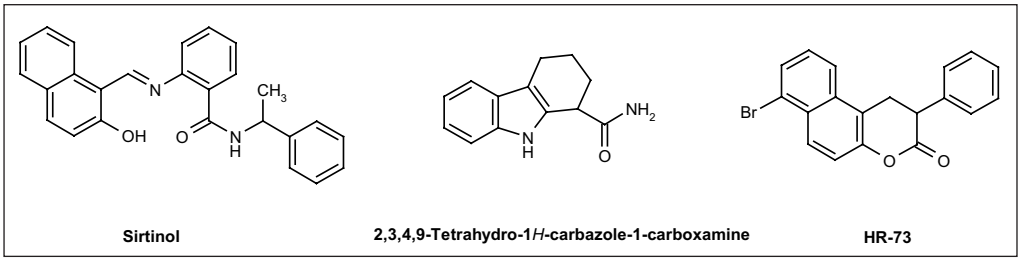
SIRT1 functions as an NAD-dependent deacetylase, removing ε-acetyl groups from protein substrate to generate ε-amino lysine residues, modulating the protein substrate function. Furthermore, the second messengers nicotinamide and 2'-*O*- and 3'-*O*-AADPR are generated. **AADPR** acetyl-ADP-ribose.

Figure 5. Substrates of SIRT1



A schematic diagram of some of the transcriptional factors with which SIRT1 interacts to produce an improved metabolic response. **CRP** C-reactive protein, **FFA** free fatty acid, **FoxO** *Drosophila* forkhead-related transcription factor, **MCP-1** monocyte chemoattractant protein-1, **PGC-1**α peroxisome-proliferator activated receptor coactivator-1α, **ROS** reactive oxygen species.

Figure 6. Structures of SIRT1 inhibitors.



The identification of some pyrroloquinoxaline-based SIRT1 activators, with approximately 2-fold activation of SIRT1 at 10 μM concentration, has been reported by Nayagam *et al* and the SAR studies were also described [48••]. The *in vitro* data from these latter compounds have been derived from SIRT1 enzyme and functional cell-based assays. However,

their utility in animal models, where the pharmacokinetics and glucose-lowering ability can be assessed, is yet to be determined.

SIRT1 modulation and metabolic control – *in vivo* evidence

Resveratrol has been evaluated in multiple models of metabolic disease. In a diet-induced obesity (DIO) model in mouse, oral daily dosing of resveratrol was demonstrated to significantly reduce glucose levels and improve insulin sensitivity [49••]. These animals also exhibited lower adiposity, increased mitochondrial mass in muscles and fat, and increased metabolic rate. Additional analysis has demonstrated that activity of the transcription factor, PGC-1α, which drives mitochondrial biogenesis, was higher in these mice, thereby providing a mechanism through which SIRT1 activation leads to such diverse physiological changes. Moreover, mice treated with resveratrol had improved exercise tolerance such that they could run twice as far (almost 2 kilometers) as those treated with vehicle. Together these data suggest that activation of SIRT1 with small molecules leads to effects very similar to those observed in animals and humans exposed to CR [50].

The positive metabolic results in the DIO model have also been confirmed in the leptin-deficient murine model and in the Zucker (fa/fa) rat [41••]. As such, resveratrol appears to improve the metabolic status in three *in vivo* models across multiple laboratories, suggesting that SIRT1 activation could indeed play a pivotal role in regulating metabolism in animals and possibly in humans. As exercise and caloric restriction are often the first lines of defense to slow down diabetes in man and because SIRT1 activation appears to mimic these natural options, it appears that drugs that can activate SIRT1 could potentially provide an alternative therapy to treating metabolic diseases.

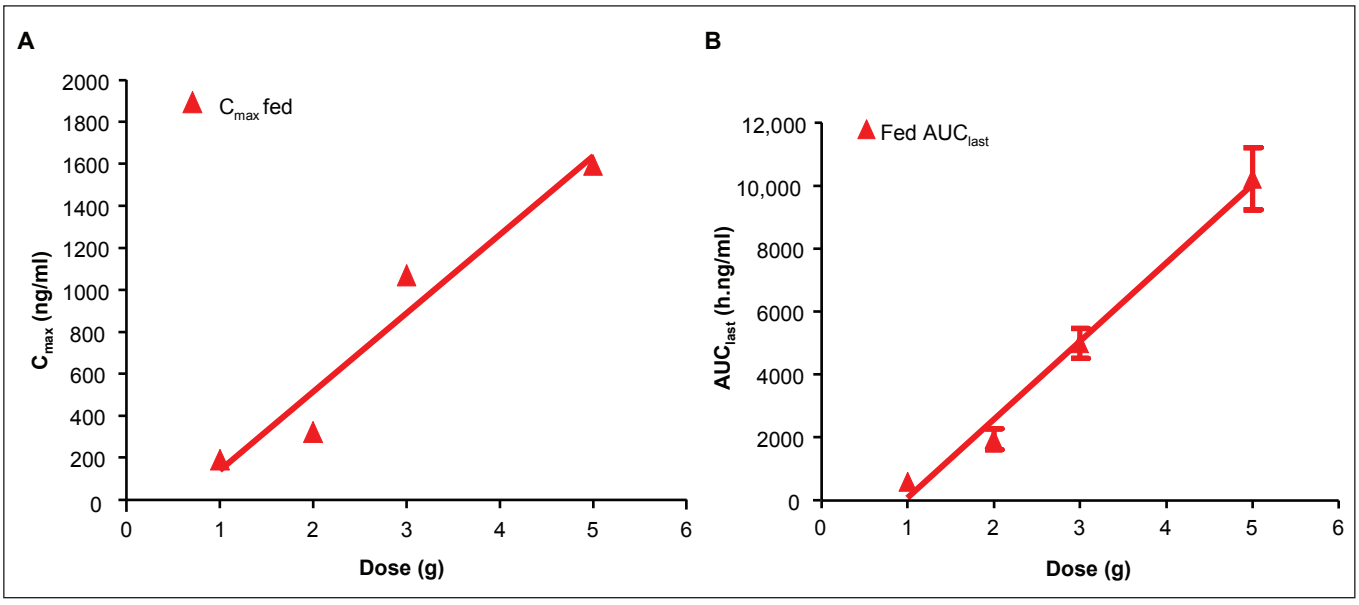
Further proof that SIRT1 activation leads to improved metabolic control is provided in studies where small-molecule SIRT1 activators, unrelated to resveratrol, elicit similar changes [41••]. With multiple and structurally different molecules activating SIRT1 *in vitro* and driving positive outcomes *in vivo*, these data strongly suggest that this mechanism is safe and may be of great therapeutic benefit. The conversion of this exciting preclinical data over the clinical hurdle is currently being attempted with resveratrol and will, it is hoped, be confirmed with additional molecules over the next few years. If the preclinical safety and efficacy effects of SIRT1 activators are confirmed in the clinical arena, they could potentially be useful in frontline therapy.

SIRT1 modulation and metabolic control – clinical evidence

The initial clinical studies with resveratrol have begun after completion of full IND-enabling safety studies in animals. These *in vivo* studies demonstrated that resveratrol was well tolerated in rodents and non-rodents even up to doses of 1 g/kg/day for 28 days. Cardiovascular, pulmonary and CNS studies in rodents and dogs showed no obvious signs of toxicity, while genetox studies (Ames test, micronucleus test etc) were all negative.

This extensive safety package allowed for advancement of resveratrol into phase I trials. Two studies have been

Figure 7. The human pharmacokinetics of SRT-501.



(**A**) This figure describes the effect of increasing oral dose of SRT-501 on maximum plasma concentration (C_{max}) in humans. Each data point is the mean from 10 subjects given a single dose of drug. (**B**) This figure shows effect of increasing oral dose of SRT-501 on maximum plasma exposure (AUC) in man. Each data point is the mean from 10 subjects given a single dose of drug.

completed in a total of 85 individuals. Results from these investigations suggest that resveratrol is both safe and well tolerated even when given at doses of up to 5 g/day for 7 consecutive days. Importantly, the pharmacokinetic coverage achieved in humans was equal to that attained in animal models of efficacy (Figure 7A and 7B) [PJ Elliott, Unpublished data]. In these phase I clinical studies, body weight and fasting glucose levels were unaffected by resveratrol, confirming preclinical data in normal animals. It is important to note that changes in these parameters are only seen when animals are given a high-fat diet, suggesting that the glucose lowering effects of SIRT1 activators should be seen in diabetic patients but hypoglycemia is unlikely to occur. As such, it is possible that resveratrol may achieve sufficient blood and tissue levels in man to observe the significant changes seen in animal models.

The next step in the development of resveratrol is currently underway in patients with type 2 diabetes. In two separate trials, resveratrol is being administered once or twice a day for 28 consecutive days to drug-naive patients. The goal of each study is to determine the safety and pharmacokinetics of resveratrol in this population to help establish dosing parameters before embarking upon more extensive trials. Data from such studies with resveratrol as a stand-alone agent for diabetes will be available in late 2008.

The final clinical study with SRT-501 is in patients with type 2 diabetes in whom metformin therapy is failing. This is a 3-month study designed to determine the effect of resveratrol on glycosylated hemoglobin A1C levels, an approvable study endpoint, in this patient population. Data from this study will also be available in late 2008. The study was initiated based on preclinical data that demonstrated that resveratrol was able to improve glucose levels and insulin sensitivity to a greater extent when given with sub-optimal doses of metformin. The hope is that the ongoing clinical trial will replicate this positive *in vivo* finding in man and provide a second option for resveratrol development as a combination therapy in such a diabetic population.

Finally, the existence of human SNP data suggests that SIRT1 might indeed be an important target for drug development [49••]. Data from the study by Lagouge *et al* demonstrated that those individuals with the SIRT1 SNPs were more likely to have lower glucose levels and improved insulin sensitivity than those without the SNPs. Moreover, the SNP subjects had lower body weight and increased metabolic rate. Although there was a positive correlation between the SNPs and energy expenditure, the SNPs do not affect the coding sequence of SIRT1. Therefore, these SNPs would not be expected to affect intrinsic SIRT1 activity. The remaining question is can this optimal human phenotype be mimicked with a SIRT1 activator such as SRT-501? The jury is still out, but there does appear to be a clear bias on the decision.

Conclusions

The seven mammalian sirtuins represent a novel family of enzyme targets that could potentially help regulate nutrient sensing and utilization, metabolic rate and ultimately metabolic disease. While for activation of SIRT3 and SIRT4 exciting preclinical data are available, the main focus of attention is SIRT1. Activation of SIRT1 leads to multiple metabolic improvements including enhanced glucose utilization, improved insulin sensitivity and increased exercise tolerance. Many of these important physiological changes are a direct consequence of increased mitochondrial biogenesis, reflecting increased activity of PGC-1α. Resveratrol represents the first in a novel class of SIRT1 activators that has proven to be safe and well tolerated in humans. Clinical trials in patients with type 2 diabetes are currently underway with resveratrol, and more potent SIRT1 activators are close behind.

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• of special interest

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