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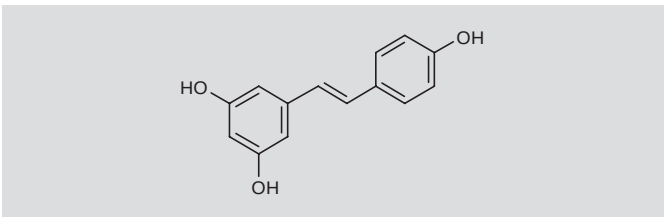
RESVERATROL/SRT501

Sirtuin SIRT1 Activator
Treatment of Type 2 Diabetes

184072
trans-Resveratrol

(*E*)-5-(4-Hydroxystyryl)benzene-1,3-diol
3,4',5-Trihydroxy-*trans*-stilbene
5-[(*E*)-2-(4-Hydroxyphenyl)vinyl]benzene-1,3-diol
InChI=1/C14H12O3/c15-12-5-3-10(4-6-12)1-2-11-7-13(16)9-14(17)8-11/h1-9,15-17H/b2-1+



$C_{14}H_{12}O_3$
Mol wt: 228.24
CAS: 501-36-0
EN: 245646

ABSTRACT

The consumption of resveratrol, a naturally occurring polyphenol found in foods, has been associated with possible health benefits. These benefits are derived, in part, from activation of NAD-dependent deacetylase sirtuin-1 (SIRT1), an enzyme thought to control the aging process in multiple species. To date, the activation of SIRT1 by resveratrol has been suboptimal due to its poor bioavailability, limiting the full treatment potential of SIRT1 activation. We have developed a novel formulation of resveratrol, SRT501, with improved bioavailability. The present report describes the safety and pharmacokinetic profile in animals and humans, as well as the glucose-lowering activity of once- and twice-daily dosing schedules of SRT501 in type 2 diabetic patients. Our results demonstrate that SRT501 was safe and well tolerated in multiple preclinical toxicity studies and in clinical trials involving healthy normal volunteers and type 2 diabetes patients. The efficacy seen in animal models of diabetes appears to be replicated in type 2 diabetic patients, and as such it shows promise as a novel treatment for diabetes.

BACKGROUND

Calorie restriction and exercise have been shown to reduce blood glucose levels in animals and humans (1-6). Indeed, diabetic patients are encouraged to modify their diets and to exercise in an attempt to reduce glucose levels (7). The benefits of calorie restriction and exercise are thought to result from an increase in the activity of the sirtuin SIRT1 (3, 8), a deacetylase proposed to mediate the beneficial effects of calorie restriction. As calorie restriction and exercise are often the frontline treatment option for newly diagnosed diabetic patients, it is possible that agents that mimic their benefits could be used early in the treatment of this disease (9).

The stilbene class of polyphenols has gained prominence in the literature principally due to the biological activities of resveratrol, which was initially isolated in 1940 (10). Resveratrol regulates the function of several genes either by altering mRNA levels or directly interacting with the translated proteins. The target genes constitute important members of signal transduction pathways, housekeeping genes/enzymes, receptors, structural proteins, etc. An exhaustive list and description of the molecular targets is covered in an excellent review by Harikumar and Aggarwal (11).

Recently, resveratrol was identified as an activator of SIRT1 (12), thereby suggesting its utility as a potential therapeutic in diseases of aging (13-17). Resveratrol exhibits activity in numerous animal model systems, including preclinical cancer models and models of inflammation (18, 19). SIRT1-dependent efficacy for resveratrol was demonstrated in an in vitro model of chronic obstructive pulmonary disease (COPD) (20). Of importance for the treatment of type 2 diabetes, resveratrol improved overall physiology, metabolic function and survival of mice on a high-calorie diet (21). Such mice had increased mitochondrial function, lower glucose and improved insulin sensitivity. In addition, resveratrol has been shown to improve exercise tolerance (17). These results were replicated in other studies where plasma glucose levels were maintained with normal insulin levels (22, 23). Finally, in a streptozotocin-induced diabetic nephropathy model, resveratrol-treated rats show attenuation of renal dysfunction (24).

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Resveratrol is known to activate SIRT1 but has limited bioavailability. Clinical experience with repeated daily administration of resveratrol up to gram doses has been published (25-32). Despite the fact that the dose and delivery vehicles varied across the different studies, these papers demonstrated that resveratrol is poorly absorbed after oral administration and undergoes rapid and significant phase II metabolism, leading to the production of four major metabolites (two monoglucuronides and two monosulfates). Several U.S. clinical trials investigating resveratrol have been completed or are currently recruiting (http://clinicaltrials.gov/), although very few have been published. Human pharmacokinetic studies with resveratrol show that it also has poor bioavailability and a short half-life. The most recent publication was a phase I dose-escalating pharmacokinetic study evaluating single doses of 0.5, 1, 2.5 or 5 g. Peak plasma levels of resveratrol at the highest dose were low and only reached 539 ± 384 ng/mL (2.4 μmol/L, mean ± SD), which occurred 1.5 h postdose. The area under the plasma concentration–time curve (AUC) values for resveratrol-3-sulfate and resveratrol monoglucuronides were up to 23 times greater than those of resveratrol (25). These data illustrate the limited plasma exposure to resveratrol after oral dosing. Due to non–dose-related pharmacokinetics, even very high doses fail to confer all the potential biological actions and therapeutic effects.

We have developed SRT501 as an improved formulation of resveratrol to potentially enhance its biological activity and therapeutic potential. The pharmacokinetics and safety of SRT501 and its activity in clinical studies in type 2 diabetes patients will now be described. It should be noted that in vitro studies were carried out only with resveratrol. However, in vivo, SRT501 is the preferred preparation of resveratrol as it has improved pharmacological properties (stability, micronization) and increased bioavailability.

PHARMACOKINETICS AND METABOLISM

From in vitro Caco-2 cell studies of intestinal permeability, we determined the P_{app} for resveratrol to be 10.6 x 10^{-6} cm/s, suggesting that absorption was between 50% and 90% of the maximal expected. Based on P_{app} values, none of the four metabolites of resveratrol (*trans*-resveratrol-3-*O*-glucuronide, *trans*-resveratrol-4′-*O*-glucuronide, *trans*-resveratrol-3-*O*-sulfate and *trans*-resveratrol-4′-*O*-sulfate) appeared to be highly permeable in this system.

Following i.v. administration of [^{14}C]-SRT501 (42.6 mg/kg, 12.7 μCi/animal), its distribution was investigated using quantitative whole-body autoradiography (QWBA). The radiolabel site was selected based on the known metabolism of resveratrol (33). QWBA analysis indicated that the compound was widely distributed, with the highest levels measured 30 min postdose and a steady decrease thereafter. The highest tissue concentrations were observed in the liver. Relatively high radioactivity concentration values were also observed in the pituitary gland, muscle and optic nerve, with detectable levels even at 9 h postdose. The brain and spinal cord demonstrated relatively low levels of radioactivity, suggesting a lack of blood–brain barrier penetration.

SRT501 is a liquid formulation of resveratrol shown to have a 3-4-fold increase in AUC and C_{max} compared to unprocessed drug in animals and man (34). Significant plasma exposure was observed after administration of SRT501 to rats and rabbits, but only limited absorption was seen in dogs.

Liver microsome and hepatocyte stability of the resveratrol in SRT501 and each of the four metabolites was measured in multiple species. Results indicated that SRT501 has intermediate clearance in the presence of human, rat and dog liver microsomes (CL_{int} = 20.2, 46.6 and 28.1 μL/min/mg protein, respectively) and high clearance in the presence of mouse liver microsomes (CL_{int} = 60.8 μL/min/mg protein). SRT501 was rapidly cleared in the presence of all species of hepatocytes, with CL_{int} values of 108, 337, 323 and 200 μL/min/10^6 cells with human, rat, mouse and dog hepatocytes, respectively. The four metabolites were metabolically stable in the presence of liver microsomes and hepatocytes of humans, dogs, rats and mice.

The cytochome P450 isoenzymes responsible for the metabolism of SRT501 and the four metabolites were investigated in bactosomes expressing the specific human isoforms. Rapid metabolism was seen in the presence of CYP1A2 (30.4% remaining after 15 min) and CYP2C19 (43.1% remaining after 30 min), and partial metabolism with CYP2C9 (86.9% remaining after 45 min) and CYP2D6 (81.3% remaining after 45 min). There was no metabolism in the presence of CYP3A4. As expected based on the liver microsome stability, the four metabolites were stable in the presence of each individual cytochrome P450 isoform. SRT501 was found to be a weak inhibitor of CYP1A (IC_{50} = 14.2 ± 2.45 μM), CYP2C9 (IC_{50} = 23.8 ± 4.56 μM), CYP2C19 (IC_{50} = 11.8 ± 2.24 μM), CYP2D6 (IC_{50} > 25 μM) and CYP3A4 (IC_{50} = 19.4 ± 4.23 μM when midazolam was used as substrate, IC_{50} = 10.9 ± 1.10 μM when testosterone was used as substrate). None of the four metabolites inhibited the cytochrome P450 isoforms (IC_{50} > 25 μM) studied.

To assess the primary routes of excretion in rats and dogs, [^{14}C]-SRT501 was administered orally at 1000 and 300 mg/kg, respectively. Fecal excretion was the major route of elimination of drug-related radioactivity in both species. Mean recoveries were 73.58% in feces and 20.75% in urine for intact rats and 60.96% in feces and 19.00% in urine for bile duct-cannulated (BDC) rats. Biliary secretion of SRT501-related radioactivity accounted for 16.53% of the radioactive dose. Mean total recoveries from all collected matrices were 94.76% for intact rats and 97.01% for BDC rats. Mean plasma concentrations of radioactivity suggested rapid absorption of drug-related radioactivity. In beagle dogs, a mean recovery of 77.97% of the radioactive dose was seen in feces. Urinary excretion of radioactivity accounted for 2.88% of the radioactive dose and cage debris samples accounted for 16.62% of the radioactive dose. The highest concentration of radioactivity in plasma (11.2 μg eq/g) was observed at 2 h postdose.

SAFETY

Published data show no adverse effects for repeated daily administration of resveratrol (20 mg/kg/day) in rats (35). However, to assess the potential human utility of high doses of SRT501, several 28-day studies were conducted under GLP conditions in rats, dogs and rabbits. Doses up to 3 g/kg given orally once daily were employed in such studies. In general, SRT501 administration was well tolerated in rats. The only finding for SRT501 (1000 mg/kg) was a slight hemolytic anemia in male rats. However, this was not associated with microscopic changes in the bone marrow or spleen, suggesting that the biological impact, if any, was minimal. Recovery animals were not anemic. The no observed adverse effect level (NOAEL) for male and

female rats was considered to be 300 mg/kg, confirming the NCI-sponsored study results previously reported (36).

In dogs, SRT501-related clinical observations were limited to abnormally colored feces (white; the color of SRT501 dosing solution) in the 100 and 300 mg/kg groups during the dosing phase of the study. There were no indications of SRT501-induced changes in weight gain or food consumption. Dosing with SRT501 at up to 300 mg/kg/day did not have any toxicological effects on heart rate, R-R interval, QRS duration or heart rate-corrected Q-T interval (Q-T_c). There were no SRT501-related changes observed during gross necropsy, organ weight or histopathological examinations. The NOAEL for male and female dogs was considered to be 300 mg/kg.

In rabbits the highest dose of SRT501 (750 mg/kg/day) required euthanasia for 5 of 8 males and 1 of 8 females due to body weight loss. Associated clinical abnormalities included discolored urine and stool abnormalities. After dosing, increases in total bilirubin and urea nitrogen were seen in high-dose females. These clinical chemistry findings were not seen after recovery. The kidneys were identified as target organs of toxicity, consistent with the literature (36), in males treated with 750 mg/kg and in females treated with 500 and 750 mg/kg. All SRT501-related histopathological findings in the kidneys were reversed following the 4-week recovery period. Oral administration of 250 and 500 mg/kg appeared to be well tolerated. Therefore, the NOAEL was considered to be 500 mg/kg in males and 250 mg/kg in females.

In a 6-month study rats were dosed orally with 0, 300, 1000 and 2000 mg/kg of SRT501. The highest dose decreased body weight and elicited discolored feces (likely dosing solution). Although total bilirubin values were elevated in the 1000 mg/kg group, the physiological significance of this increase is unclear due to the absence of any adverse histopathological changes, and these levels returned to normal after the recovery period. There were no other physiologically relevant changes in the other groups at the end of the dosing period. As such, the NOAEL was 300 mg/kg.

A 6-month study in rabbits was completed using once-daily doses of SRT501 (100, 300 and 500 mg/kg). There was no drug-related mortality observed during the study. Importantly, there were also no apparent treatment-related clinical abnormalities. At the end of dosing, small elevations in erythrocytes (~9-10%), hemoglobin (~8-11%) and hematocrit (~7-8%) were observed at the highest dose but the physiological relevance of these alterations is unclear. The most notable clinical chemistry parameter changes included modest increases in total protein, albumin, GGT, globulin, glucose, urea nitrogen, indirect bilirubin and calcium, and decreases in potassium in animals treated with 500 mg/kg, but these were not present in the recovery animals. As such, the NOAEL in this study was 500 mg/kg.

A segment II reproductive toxicity study was conducted in rats using SRT501 (300, 1000 and 3000 mg/kg) or vehicle administered orally once daily on days 7 through 17 of presumed gestation. At the highest dose level of 3000 mg/kg, 5 of 25 animals were sacrificed due to adverse clinical conditions. No effects on the fetus were noted in this study. As such, the maternal NOAEL of SRT501 was 300 mg/kg and the developmental NOAEL was 1000 mg/kg.

The cardiovascular and respiratory safety of SRT501 was studied in telemetered dogs. Animals received a single oral dose of 0, 300 or 1000 mg/kg SRT501. All doses were well tolerated. No significant effects on hemodynamic parameters, respiratory rate, core body temperature and arterial blood gases were observed during the study. There were no electrocardiogram abnormalities or Q-T/Q-T_c prolongation. The acute neurotoxicity of SRT501 (300 and 1000 mg/kg) was also assessed in rats. Both doses were well tolerated after acute oral administration and did not elicit any abnormal central nervous system (CNS) effects.

SRT501 was negative in the Ames test and in the mouse lymphoma and mammalian micronucleus tests. As such, SRT501 does not appear to have any genotoxic liability.

Following the evaluation of preclinical pharmacology and safety, testing of SRT501 was advanced to healthy normal volunteers. SRT501 was shown to be safe and well tolerated when given to healthy subjects daily for 7 consecutive days. Importantly, no evidence of hypoglycemia, weight gain or edema was observed in either study. Subsequently, the safety, efficacy and pharmacokinetic profile of SRT501 were evaluated in patients with type 2 diabetes.

CLINICAL STUDIES

Two hundred and fourteen drug-naïve male type 2 diabetes patients (aged 18-62) were recruited into two separate trials (003 and 006). The first trial evaluated a once-daily (q.d.) treatment regimen and the second trial evaluated a twice-daily (b.i.d.) regimen of SRT501. Subjects were randomized to receive placebo, SRT501 at a total daily dose of 2.5 g or SRT501 at a total daily dose of 5.0 g. While the primary focus of each study was the safety and pharmacokinetic profile of SRT501, measures of glycemic control were recorded, including fed and postprandial glucose and insulin levels. Subjects also underwent oral glucose tolerance testing (OGTT). Baseline demographics in both studies were similar with no prestudy group bias. Baseline glucose and insulin samples were collected prior to dosing on day 1 of the clinical study and were collected again at week 4. Following 28 days of q.d. or b.i.d. dosing, there were no dose-related or dose-limiting effects on physiological, clinical chemistry, hematological, cardiovascular, liver and renal parameters in any treatment group. Adverse events were generally mild in nature and reversible. Pharmacokinetic testing revealed that maximum plasma levels of SRT501 (t_{max}) were reached within 4 h. This was unchanged with 28 days of dosing. The highest concentrations (C_{max}) were also unchanged during the studies, indicating no evidence of drug accumulation or obvious changes in how the drug was metabolized/excreted. Moreover, the C_{max} data were dose-proportional, which was confirmed using AUC (area under the curve) analysis. With q.d. dosing of SRT501, fasting and postprandial glucose levels were reduced compared to placebo, although this failed to reach significance. Results from the b.i.d. study (Fig. 1A) showed that 5.0 g/day lowered fasting glucose in a statistically significant manner (P = 0.04), with a trend observed with 2.5 g/day (P = 0.18). SRT501 (5.0 g/day) reduced postprandial glucose (P = 0.0018), with a trend observed with 2.5 g/day (P = 0.0656) (Fig. 1B). Fasting insulin (Fig. 1C) and HbA1c levels were unaffected in either trial. Postprandial insulin was also unaffected by q.d. dosing, but results from b.i.d. administration showed that SRT501 lowered postprandial insulin, albeit in a non-statistically significant manner (P = 0.08) (Fig. 1D). The OGTT data from both trials demonstrated an improve-

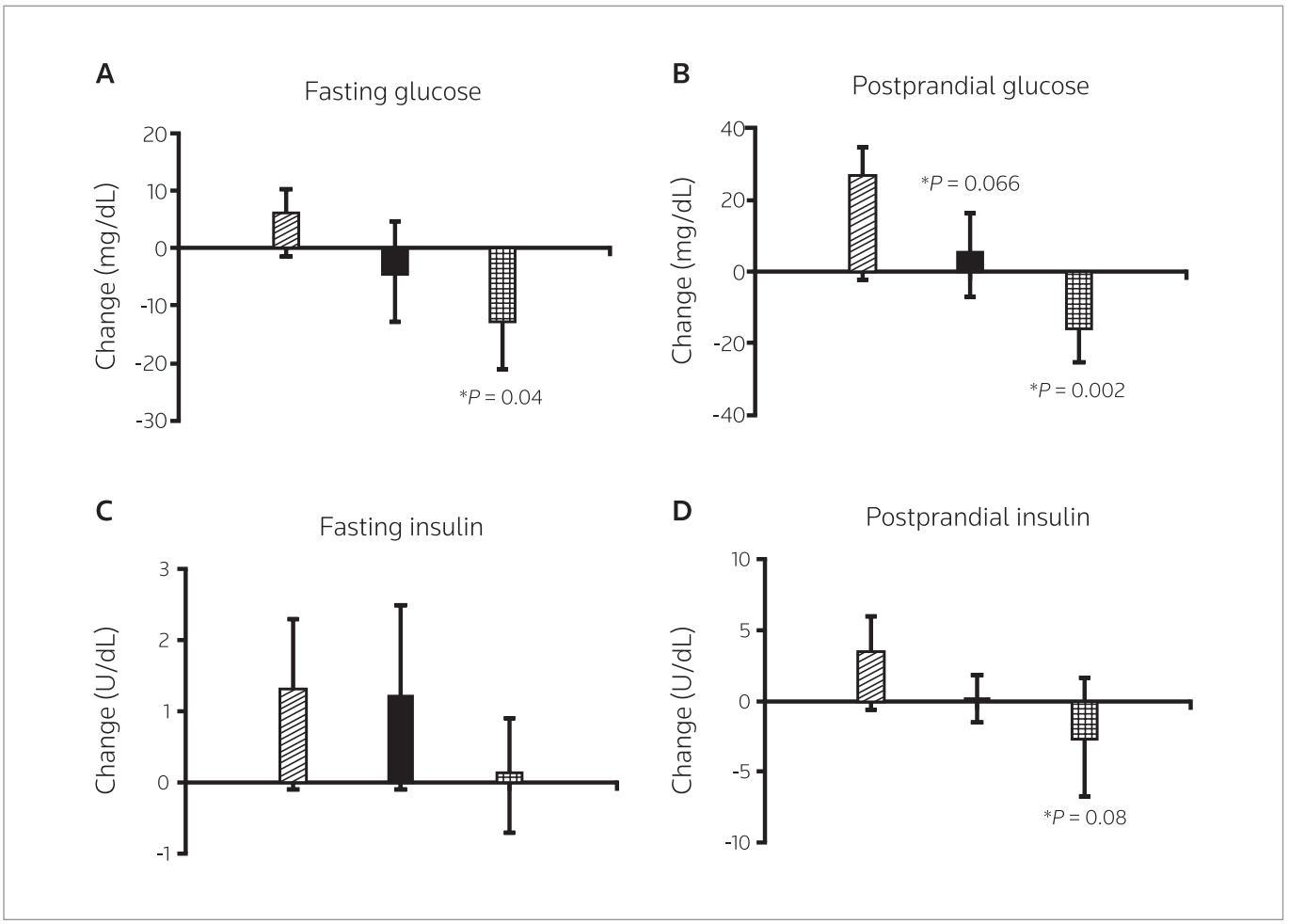

Figure 1. Effects of SRT501 on fasting and postprandial glucose and insulin levels in type 2 diabetics. Data were collected from separate groups of subjects (n = 31-36) in each study. Diagonal bars represent placebo-treated subjects; solid bars represent data from subjects treated with 2.5 g/day SRT501; crosshatched bars represent data from subjects treated with 5.0 g/day SRT501. Glucose and insulin levels were collected from subjects after an overnight fast or 2 h after a meal (postprandial). Data were analyzed using pooled variance t-tests (mean ± SD). Baseline fasting glucose (mg/dL) = 190 ± 65. Baseline fasting insulin (U/dL) = 6.7 ± 2.8. Baseline postprandial glucose (mg/dL) = 241 ± 93. Baseline postprandial insulin (U/dL) = 19.0 ± 15.3.

ment with SRT501 treatment. With q.d. dosing the effects failed to reach statistical significance, but with b.i.d. dosing both doses of SRT501 were associated with a statistically significant improvement in glucose excursion during this test. The improvement was evident after 1.5 h and this effect was observed until the last time point (3 h; data not shown).

CONCLUSIONS

Considering the predicted normal biological variance within the endpoints measured, the positive glucose-lowering effects demonstrated with SRT501 are encouraging. As such, this is the first report of a SIRT1 activator improving glucose levels in man. These studies showed no evidence of drug accumulation following SRT501 administration and dose proportionality was observed, indicating that SRT501 may have utility in the treatment of multiple disease states modulated by SIRT1 activation. Animal data show that SRT501 lowers glucose levels and improves insulin sensitivity (9), and may be at least additive when given with metformin (unpublished results). As such, clinical studies have been initiated to explore the utility of SRT501 in combination with the standard of care metformin, as well as in cancer. Additionally, more potent activators of SIRT1 have entered clinical development for the treatment of type 2 diabetes and other diseases of aging.

SOURCE

Sirtris, a GlaxoSmithKline Company (US).

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