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A randomized clinical study assessing the effects of the antioxidants, resveratrol or SG1002, a hydrogen sulfide prodrug, on idiopathic oligoasthenozoospermia

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ABSTRACT

Objective: To determine whether subjects suffering from oligoasthenozoospermia would benefit from antioxidant treatment with resveratrol, a natural-occurring polyphenol, and hydrogen sulfide. **Methods:** A randomized controlled clinical trial involving 54 men with Oligoasthenozoospermia. We randomly assigned resveratrol (n=18), SG 1002 (n=18), and placebo (n=18) for 75 days. Sperm analysis was performed after treatment. Statistical analysis was made with chi square test. **Results:** When compared to the placebo treated group, SG1002 treatment led to an increase in sperm concentration (11.18×10^6 *vs.* 17.01×10^6, $P < 0.05$), sperm motility (10.06×10^6 *vs.* 20.06×10^6, $P<0.05$) and motile forms recovery (0.33×10^6 *vs.* 1.62×10^6, $P<0.05$). Resveratrol treatment did not significantly affect any of the parameters. **Conclusions:** SG1002 may reverse oligoasthenozoospermia. It seems to be more potent antioxidant than resveratrol. This findings need be supported by further clinical investigation.

1. Introduction

Approximately 10% of all couples are infertile, or incapable of conceiving during a 12 month period[1], with male infertility accounting for or contributing to approximately 50% of these cases[2]. Although there are a number of problems that can lead to male infertility, observed alterations in the concentration and/or motility and/or morphology of semen, collectively termed oligoasthenozoospermia, account for approximately 25% of all cases of male infertility[3]. Despite numerous clinical studies, successful therapeutic treatments of oligoasthenozoospermia have not yet been developed and assisted reproduction remains the standard of care for infertile couples[4].

Reactive oxygen species (ROS) play an important role in normal spermatogenesis, maintaining a delicate balance in the male reproductive tract of healthy men and are necessary for capacitation, acrosome reaction and fertilization[5,6]. However, an increase in ROS leads to oxidative stress and high levels of ROS[7–9] or corresponding impaired antioxidant capacity[10–12] can lead to sperm dysfunction. ROS are a group of substances with highly reactive molecules that oxidize and modify biomolecules that are nearby. They react almost immediately with any nearby substance, causing cell damage[5]. Semen contains antioxidant enzymes of high molecular weight (superoxide dismutase, catalase and glutathione peroxidase) and a deficiency of these is also associated with alterations in male fertility[7].

Reviews of numerous clinical studies undertaken to reduce levels of ROS found that use of antioxidants may increase the likelihood

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of live birth and pregnancy rates and improve the results of assisted reproduction techniques[5, 13]. The role of dietary antioxidants has also been implicated in male fertility. Mendiola *et al*[14] showed that a low intake of dietary antioxidants has a deleterious effect on sperm quality, and that there was an improvement in sperm count and motility in patients who consumed a diet high in antioxidants, especially vitamin C. In addition, folic acid and zinc sulfate administered for 26 weeks led to an increase of up to 74% in the total sperm count in a group of subfertile men[15]. However, in another study, although antioxidants led to a decrease in DNA fragmentation, the authors also reported an increase in sperm decondensation which they felt might interfere with sperm preimplantation[16]. Finally, one of the leaders in the study of oxidative stress and oligoasthenozoospermia, Dr Ashok Agarwal, upon a thorough review of the clinical literature concluded that further well-controlled, randomized studies are needed to assess the safety and efficacy of antioxidants. Thus, the objective of the present study was to determine initially, in a small number of patients, whether two antioxidants, resveratrol and SG1002, both know to be very safe, could alter sperm count, mobility or abnormalities.

Resveratrol (3,5,4´-trihydroxystilbene) is a polyphenol with a wide variety of biological functions including antioxidant activity[18]. A diet that includes resveratrol in its monomeric form or foods that contain this substance, have been associated with improvement of diverse biological functions, such as, cardioprotection, anticancer activity, anti-inflammatory effect, estrogenic/antiestrogenic properties, and modulation of cell signaling. Although resveratrol has been reported to have excellent antioxidant properties[18] other than testing it as a cryopreservative, few studies have assessed its ability to improve oligoasthenozoospermia. In rodent models of dysfunctional spermatogenesis, resveratrol has been show to improve sperm count[19–21], including reversing the process of testicular damage, increasing testicular weight, causing seminiferous epithelium differentiation, and restoring the physiological process of spermatogenesis[22]. Yet, despite the encouraging animal studies and known safety profile, resveratrol has not been studies for its ability to improve oligoasthenozoospermia in patients.

Hydrogen sulfide, a gaseous transmitter found in the blood acts as a very strong antioxidant by virtual of its rapid breakdown to HS molecules, which are very powerful one electron chemical reductants[23,24]. For this reason, hydrogen sulfide prodrugs have been proposed for use in treating a number of diverse medical problems[25], ranging from cardiovascular disease[26] to cancer[27]. In addition to its antioxidant properties, potent anti-proliferation/cytotoxic and anti-inflammatory activities have been proposed. The active compounds/metabolites associated with these effects are thiosulfinates, isothiocyanates, trisulfides, tetrasulphides, and polysulfides. Although their mechanism of action is not entirely clear, it appears that they act in combination to produce oxidative reactions, decomposition of highly hydrophilic species, inhibition of metalloenzymes, imbalance of metal homeostasis, membrane integrity, and interference of different cell signaling pathways[28,29].

HS has recently emerged as a critical physiological gaseous signaling molecule that is produced enzymatically in al mammalian species at low micromolar levels via the action of cysteine metabolic enzymes. Recently it has been demonstrated that HS protects acute myocardial ischemia/reperfusion injury via antiapoptotic effects mediated by phosphatidylinositol-3-kinase/Akt, protein kinase C, and extracellular signal-regulated kinase 1/2 pathway, as well as antioxidant actions via the activation and translocation of Nuclear-factor-E2-related factor-2 to the nucleus, including an increase in antioxidant response element-related antioxidants. Moreover, previous experimental studies suggest that HS augments angiogenesis under ischemic conditions both *in vitro* and *in vivo*. H2 also exhibits potent anti-inflammatory actions and modulates mitochondrial respiration in part by reversible inhibition of cytochrome c oxidase.

Although the male factor in infertility is a complex area of study and treatment, free radicals and antioxidants appear to play a very important role in sperm maturation and activity. The main objective of this study is therefore to evaluate and compare the effects of resveratrol and a hydrogen sulfide prodrug, SG1002, on sperm parameters in idiopathic oligoasthenozoospermia.

2. Material and methods

2.1. Investigational products

Resveratrol was obtained from Nuevas Alternativas Naturales Thermafat, S.A. de C.V. (Monterrey, Mexico). SG1002 was provided by Nuevas Alternativas Naturales Thermafat, S.A. de C.V. (Monterrey, Mexico). SG1002 is a proprietary hydrogen sulfide prodrug composed of alpha sulfur, sodium sulfate, sodium thiosulfate and trace amounts of polythionates obtained through a single synthesis. In animal studies (unpublished data communicated by Nuevas Alternativas Naturales Thermafat, S.A. de C.V.), SG1002 resulted in high circulating levels of hydrogen sulfide and excellent antioxidant properties. The presence of the highly polar, ionic species found on the surface of the microcrystalline powder are believed to be responsible for SG1002's high bioavailability and the corresponding circulating levels of hydrogen sulfide. Since all the components of SG1002 are natural products known to be safe for use in man, like resveratrol, safety is not a concern.

2.2. Clinical design

This randomized, double blind study enrolled 54 patients with a diagnosis of idiopathic oligoasthenozoospermia. All subjects gave signed written informed consent and the study was approved by the Ethics Committee of the Universidad Autónoma de Nuevo León University Hospital (registration number BR09-001). Subjects were randomly assigned to either placebo, resveratrol or SG1002 treatment groups.

Patients who participated in this study were recruited from the Reproductive Biology Clinic of the University Hospital from July 2009 to September 2010. We included patients between the ages of 20 and 45 years of age with a diagnosis of idiopathic oligoasthenozoospermia. The diagnosis of oligoasthenozoospermia was reached by performing two semen analyses on different dates with an interval of three weeks between them. All infertile patients with normal findings on semen analysis, patients who were chronic smokers or those who had been taking antioxidants in the last 6 months prior to study entry were excluded from the study. Patients with chronic degenerative diseases such as diabetes or high blood pressure or with hormonal abnormalities were also excluded.

At the screening visit, patients were consented, a complete medical history, physical examination and semen analysis was obtained for all potential subjects. Those subjects meeting the inclusion/exclusion criteria underwent a second semen analysis after sexual abstinence of 3 to 5 days, which was used as the baseline data (sample 1). Subjects were then randomly assigned to one of the three treatment groups (18 subjects/group), and provided with a 75-day supply of either placebo (750 mg microcrystalline cellulose), resveratrol (25 mg resveratrol and 725 mg microcrystalline cellulose) or SG1002 (750 mg SG1002) capsules. Bottles and capsules for each treatment were identical and identified by a code unknown to the researchers or subject. Each subject was also provided with a patient log form to record adverse events, including the type and frequency. Subjects were instructed to take two capsules daily and record and deviations.

Thirty days following randomization, subjects either visited the clinic or participated in a follow-up phone call to document adverse effects and adherence to treatment.

The final visit was conducted 75 days after starting treatment; subjects returned to the clinic, with a window period of +/- 2 or 3 days depending if there was a weekend or holiday going on. Adherence was verified by review of log books and counting the remaining capsules and adverse effects were recorded. Prior to this visit, subjects were asked to abstain from sex for 3 to 5 days. A sperm sample was collected at this visit for post-treatment semen analysis (sample 2).

2.3. Sperm analysis

Sperm concentration expressed in sperm cells x 10^6 per milliliter and motility (obtaining only the percentage of progressive sperm cells A + B, from the total concentration) was determined using an automated IVOS (Integrated Visual Optical System) device and manually confirmed by lab technicians, blinded to the treatment group. The morphology of each semen analysis was manually assessed, according to Kruger criteria. Mobile froms recovered (MFR) were assessed after semen processing.

2.4. Statistical analysis

Traditional descriptive data, such as measures of central tendency (means, median and mode) and in the case of quantitative variables, measures of dispersion (variance, standard deviation and coefficient of variation) were studied for each variable, together with the frequencies observed in qualitative variables. Statistical analysis was performed using *Chi* square test, comparing samples from the resveratrol or SG1002 groups to the placebo group following treatment at a confidence interval of 95%.

3. Results

Fifty-four subjects met the inclusion/exclusion criteria and were randomized into one of three treatment groups. All subjects had asthenozoospermia as characterized by less than 25% type A sperm motility or less than 50% type A + B sperm motility, and oligozoospermia as determined by a concentration of less than 20 million sperm per milliliter, as defined by the World Health Organization[30]. Forty-seven of the randomized subjects completed the protocol.

Of the seven subjects who did not complete the study (3 from the placebo group, 2 from the resveratrol treatment group and 2 from the SG1002 treatment group), none returned for follow-up visits and therefore no data on sperm count, motility or abnormality was available and an intent to treat analysis could not be carried out. Four of these subjects were lost in follow-up while the other three withdrew due to unpleasant smelling sweat (SG1002 treatment group), nausea and flatulence (SG1002 treatment group), and inconvenience (SG1002 treatment group).

The log book from all subjects completing the study were reviewed and adverse events recorded. None of the differences in adverse events, however, were statistically significant nor were any deemed to be clinically significant.

The primary objective of this study was to determine whether the use of antioxidants, either resveratrol or SG1002 could effect sperm number, motility or reduce abnormalities. Semen was

therefore analyzed prior to initiating treatment and on the final day of treatment. Baseline data is reported only for those completing treatment as the baseline measurements from the subjects that dropped out of the study did not differ significantly from those completing the protocol.

Sperm baseline concentration ranged from 0.5×10^6/mL to 19.9 $\times10^6$/mL, with a mean concentration of 10.84×10^6/mL. The mean baseline concentration was 10.7×10^6/mL,10.9×10^6/mL and 11.2× 10^6/mL for the placebo, resveratrol and SG1002 treatment groups, respectively, which were not significantly different. Following the 75 day treatment period, sperm concentration did not change for the placebo group or the resveratrol treatment group. However, SG1002 significantly increased sperm concentration, 17.1 x 10^6/mL, when compared to the placebo group ($P< 0.05$) (Table 1).

A + B type sperm motility was not significantly different at baseline, although the A + B motility of the placebo group (8.3%) was noticeably lower than those of the resveratrol (14.4%) and SG1002 (13.4%). Treatment did not significantly change A + B motility for the placebo group or the resveratrol group. SG1002 treatment did significantly increase A + B sperm motility when compared to the placebo group following treatment ($P< 0.05$) (Table 1).

In addition to A + B sperm motility, mobile form recovery (MFR) was also assessed pre- and post-treatment. MFR post-sperm capacitation was (0.38×10^6), (0.40×10^6), and (0.58×10^6) for the placebo, resveratrol and SG1002 groups prior to treatment. Although MFR was higher for the SG1002 group, the difference was not significant when compared to the placebo group. Only the SG1002 treatment resulted in a significant increase in MFR ($P<0.05$) (Table 1).

Sperm morphology was similar between all treatment groups at baseline (30.1%-32.1%). Morphology did not significantly change following treatment with either resveratrol or SG1002 (Table 1).

Table 1
Characteristics of the first and second samples in the three groups.

Characteristic	First Samlpe			Second Samlpe		
	Hydrogen sulfide prodrug	Resveratrol	Placebo	Hydrogen sulfide prodrug	Resveratrol	Placebo
Sperm concentration	11.02×10^6	10.90×10^6	10.70×10^6	17.01×10^6	11.56×10^6	11.18×10^6
Motility A+B (%)	13.43	14.43	8.33[a]	20.06	16.81	10.06
Normal morphology (%)	31.60	32.06	30.06[a]	36.30	34.18	30.40
MFR	0.58×10^6	0.40×10^6	0.38×10^6	1.62×10^6	0.487	0.34×10^{6a}

Note: Statistical analysis was performed using x^2; MFR: Mobile Forms Recovery, post sperm capacitation; no statistically significant differences were found between placebo and hydrogen sulfide prodrug / between placebo and resveratrol; [a]$P<0.05$ comparing sulfide prodrug with resveratrol for the second sample.

4. Discussion

The male factor in infertility causes about 25% to 30% of cases of infertile couples, and up to 30% of abnormalities can be detected in both partners[15, 16]. Many aspects of male infertility are poorly understood. Medical treatment for these patients is largely empirical and often fruitless. That is why pharmacological management of male infertility is often overlooked and couples are treated directly with assisted fertility procedures. However, the results of this study allow us to propose therapy with antioxidants such as hydrogen sulfide prodrug as a valid method for improving spermatogenesis in carefully selected patients. This is the first prospective, controlled, randomized, double blind clinical trial that shows that hydrogen sulfide prodrug therapy improves some seminal parameters.

All study subjects who did not comply with medication given as prescribed, who discontinued the drug or were hypersensitive to it were eliminated. An increase in sperm concentration was observed in the three groups, with the hydrogen sulfide prodrug group being higher; this was the only group with a statistically significant increase. Probably in a sample with a greater number of patients, a statistically significant difference would also become apparent in the resveratrol group. These findings demonstrate and confirm the data obtained in other studies where antioxidant therapy appears to be effective in the management of patients with oligoasthenozoospermia[17]. However, it is noteworthy that our study is the first that uses resveratrol and hydrogen sulfide prodrug as antioxidants in the management of male oligostenozoospermia, although their antioxidant potential and beneficial effect in general health have been widely described in other work[13, 18].

There have been other studies that report an increase in sperm concentration in men with oligoasthenozoospermia in which recombinant follicle-stimulating hormone (r-FSH) was used for periods similar to this study (3 months) with variable results that show no significant increase in the sperm count in men with oligozoospermia and a failure in improving pregnancy rates[19]. There are also studies that show conflicting results where therapy

with recombinant r-FSH, injected alternately for periods of three months, can significantly increase sperm production in patients with idiopathic oligozoospermia[20]. The patients in our study received treatment for a period of 75 days, and it was shown that this is a reasonable period in order to find favorable results in sperm counts with oral treatment, compared with parenteral administration, a more uncomfortable method used in treatment with r-FSH.

In this study we found that supplementation with hydrogen sulfide prodrug resulted in increased sperm motility both pre-and post-capacitation, in contrast to previously published studies, such as that conducted by Donnelly *et al*, which reported that supplementation with antioxidants did not improve sperm motility *in vitro*. This difference can be attributed to the fact that this study used ascorbic acid and -tocopherol as antioxidants[21]. It is known that even though the hydrogen sulfide prodrug species are less known and studied, their antioxidant effect is much greater than ascorbic acid and -tocopherol; therefore it is feasible that in our study, besides the fact that it was performed *in vivo*, we also obtained satisfactory results with regard to motility. We also observed that sperm morphology can be improved after treatment with hydrogen sulphide donors, and although there are not many studies that focus on improving sperm morphology, we believe this result is justified because the antioxidant potential of this substance is capable of reducing the amount of reactive oxygen species with oxidative potential at the testicular level, decreasing cell damage[22].

There have been many studies about resveratrol, its antioxidant potential and its beneficial effects on the body. Our study is the first where this substance has been used to improve sperm quality in humans. Works have been reported showing that resveratrol has a protective effect against oxidative damage induced by cryopreservation of human semen, but it was not proved to be able to restore the reduction in motility caused by this process [23], suggesting that it may not have a beneficial effect on increasing motility. This fact was also observed in our study, since supplementation with this substance for 75 days did not significantly increase concentration or sperm motility. However, in the literature there are studies that have attributed beneficial effects on sperm parameters in animals, saying that the use of this substance protects the sperm against DNA damage and apoptosis induced by exposure to environmental toxins in rats[24]. It has also been published that the administration of this natural antioxidant increases the sperm count in healthy rats[8], and restores spermatogenesis after testicular damage induced by 2,5-hexanedione in rats, a toxic substance inducing testicular damage[9]; thus further studies are needed with a larger sample of patients to adequately elucidate the possible benefit of resveratrol in human sperm parameters.

With regard to the tolerability of the substances used, it is noteworthy that only one patient reported nausea and flatulence during the first three days, making him decide to stop taking the drug. This patient belonged to the hydrogen sulfide prodrug group. We conducted a comprehensive search of Pubmed, and Medline, using the keywords: “sulfur donors”, “collateral effects”, “nausea” and “flatulence” and found no similar effects in other studies, so we assume that this patient had a degree of intolerance to hydrogen sulfide prodrug. This finding was not reported in any other patient group. Another patient complained of strange smelling sweat, so he stopped using the drug. This patient belonged to the hydrogen sulfide prodrug group. We searched PubMed and Medline using the keywords: “sulfur donors”, “collateral effects” and “smelly sweating”, trying to associate the words, without obtaining a reference which reported the same symptoms, thus we can assume that this isolated finding is due to other causes such as exercise or excessive sweating or specific patient characteristics, not just use of the drug.

This study demonstrates that resveratrol and hydrogen sulfide prodrug are antioxidants that are well tolerated by the human body, without developing significant adverse effects at the doses used. We also showed that the prodrug hydrogen sulfide is an antioxidant that can increase the sperm count, motility, normal morphology, and MFR post-capacitation. It was found that resveratrol does not increase the sperm count or motility and that it also does not have a beneficial effect on sperm morphology.

This study is unique in its kind and although the results cannot be compared with others, they are promising enough to motivate further study of these substances.

Declare of interest statement

The authors of this manuscript certify that no conflict of interest of any kind exists.

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