BJCP British Journal of Clinical Pharmacology

DOI:10.1111/j.1365-2125.2009.03450.x

Tolerability and safety of GS-101 eye drops, an antisense oligonucleotide to insulin receptor substrate-1: a 'first in man' Phase I investigation

Hermann Kain, David Goldblum, Bernard Geudelin,[1] Eric Thorin[2] & Christoph Beglinger[3]

Eye Clinic, University Hospital Basel, Basle, [1]Mediante GmbH, Eptingen/Basel and [3]Clinical Research Centre, University Hospital Basel, Basel, Switzerland, and [2]Montreal Heart Institute, Université de Montréal, Montréal, Québec, Canada

Correspondence
Professor Eric Thorin, Université de Montréal, Montreal Heart Institute, Department of Surgery, 5000 rue Bélanger, Montréal, Québec, H1T 1C8, Canada
Tel: + 514 376 3330
Fax: + 514 376 1355
E-mail: eric.thorin@umontreal.ca

Keywords
antiangiogenic, cornea, insulin receptor substrate-1, pathologic neovascularization

Received
2 November 2008

Accepted
15 April 2009

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Corneal proliferative angiogenesis is an orphan disease leading to cornea loss.
- Today's therapeutic approach is not clearly established, although essentially based on topical corticosteroids.
- There is therefore a need for new therapeutic alternatives specifically targeting angiogenesis.

WHAT THIS PAPER ADDS

- Preclinical data demonstrated the efficacy of GS-101, an antisense oligonucleotide inhibiting insulin receptor substrate-1 expression, at inhibiting experimental corneal angiogenesis.
- This study demonstrates the excellent safety and tolerability profile of GS-101 applied as eye drops three times daily in this 'first-in-man' study.
- Upon validation in human of its immediate benefit, GS-101 would offer an alternative to antivascular endothelial growth factor therapies in the treatment of corneal angiogenesis.

AIMS

GS-101 (GeneSignal, Epalinges, Switzerland) is an antisense oligonucleotide that inhibits the expression of the scaffold protein insulin receptor substrate-1 (IRS-1). Inhibition of IRS-1 results in the prevention of neovascular growth and was shown to prevent the angiogenic process in preclinical *in vitro* and *in vivo* experiments. There is therefore a strong therapeutic rational for targeting angiogenesis in pathological neovascularization. We aimed to investigate the safety, tolerability and bioavailability of GS-101 eye drops.

METHODS

This was a Phase I open-label study. The investigation was performed in two steps. Local ocular tolerability was first assessed with the application of one single low dose in one eye. After no signs of intolerance were observed in the subjects, the dose escalation phase of the study was initiated, and the remaining subjects received three times daily escalating doses of GS-101 in one eye for 14 days.

RESULTS

The 14 healthy volunteers tolerated well 14 days' continued use of escalating doses of GS-101 from 43 to 430 μg per day. Other than itching, experienced also in the control eye by one subject and determined to be unrelated to the study treatment, no signs of intolerance were observed.

CONCLUSIONS

The tolerability profile obtained from this study suggests that GS-101 is safe for human use. Further clinical evaluations in diseases related to abnormal angiogenesis are being targeted. In particular, the neovascularization-related orphan indications of corneal graft rejection, retinopathy of pre-maturity and neovascular glaucoma are currently under Phase II clinical investigation and are showing promising results.

Introduction

Angiogenesis is a fundamental aspect of many normal and abnormal biological processes. Wound healing, embryonic development and the menstrual cycle are examples of normal angiogenesis. Both excessive and insufficient angiogenesis can be pathological. Insufficient angiogenesis can lead to stroke, heart disease, ulcers, infertility or scleroderma, whereas excessive angiogenesis is at the root of rheumatoid arthritis, psoriasis, cancer and certain forms of blindness [1–4].

Insulin receptor substrate 1 (IRS-1) is a cytoplasmic docking protein that functions as an essential signalling intermediate downstream of activated cell surface receptors, including insulin, insulin-like growth factor 1 (IGF-1), prolactin, growth hormone, vascular endothelial growth factor (VEGF) receptors, members of the integrin receptor family, and select cytokine receptors [5–9]. IRS-1 was originally identified as a substrate of the insulin receptor, and it has been predominantly studied for its role in metabolic signalling [9]. The role of IRS-1 in angiogenesis stems from the fact that insulin and IGF systems have been implicated in several vascular diseases, including angiogenesis [10]. IRS-1 could mediate the regulation of VEGF [11] or other proangiogenic cytokines [12]. Mice knockout for IRS-1 gene expression develop less neovascularization [13]. There is therefore a strong therapeutic rationale for targeting IRS-1 inhibition where excessive angiogenesis is part of the pathological process [14–16].

GS-101 (GeneSignal, Epalinges, Switzerland) is a 25mer phosphorothioate antisense oligonucleotide with a molecular mass of 8036 Da (5′-TATCCGGAGGGCTCGC CATGCTGCT-3′). GS-101 inhibits the expression of IRS-1 [17]. This inhibition of IRS-1 results in the prevention of neovascular growth and has been reported to prevent the angiogenic process in preclinical *in vivo* and *in vitro* experiments [17, 18]. Specifically, the neovascularization-related orphan indications of corneal graft rejection, retinopathy of pre-maturity and neovascular glaucoma are being targeted as initial clinical applications of GS-101. This safety, tolerability and bioavailability investigation is the first step in the development of GS-101 as a novel antiangiogenic therapeutic agent.

Materials and methods

The study protocol and the patient informed consent form were approved by an independent Ethics Committee (Ethische Kommission Beider Basel) and by the Swiss Health Authorities (Swissmedic) prior to starting the study. The study was conducted according to the Declaration of Helsinki III and in accordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects as laid down by the Council for International Organizations of Medical Sciences in collaboration with the World Health Organization and the Good Clinical Practice guideline CPMP/ICH/135/95.

Good manufactory practice batches of GS-101 were provided by the company Gene Signal Interational SA (Epalinges, Switzerland ; patents US#7,417,033; EP#1,409,672; CA#2,451,874). GS-101 was re-suspended in sterile saline solution (0.9% NaCl) daily at room temperature and was used as such. Each drop of GS-101 solution was calibrated to a volume of 50 µl.

Protocol design

This 'first-in-man' Phase I open-label study was designed to investigate the safety, tolerability and bioavailability of GS-101 eye drops. The investigation was performed in two steps. Local ocular tolerability was first assessed in three subjects with the application of one drop (14.3 µg per drop) three times per day in one eye only, for a total dose per day of 43 µg. After no signs of intolerance or adverse events were observed in these subjects after 3 days, the same application was applied a second time, and the subjects were followed for another three consecutive days.

After no signs of intolerance or adverse events were observed in these subjects after the second 3-day observational period, the dose-escalation phase of the study began. The dose regimen was as described in Figure 1. Twelve volunteers received three times daily escalating doses of GS-101 in one eye over a 14-day period. The study drug was administered by eye drops of 50 µl in one eye only; the second eye was monitored as the control eye. The eye assigned to treatment was not randomized but chosen by the volunteer. The control eye did not receive the vehicle (sterile NaCl 0.9% solution) following the scientific committee recommendations.

As presented in Figure 1, the first three doses were administered for 2 days before moving to the second dose. The fourth dose was therefore administered for the last 7 days of the study. All volunteers received three drops per day of 14.3 µg (43 µg day^{-1}, days 1 and 2), 28.7 µg (86 µg

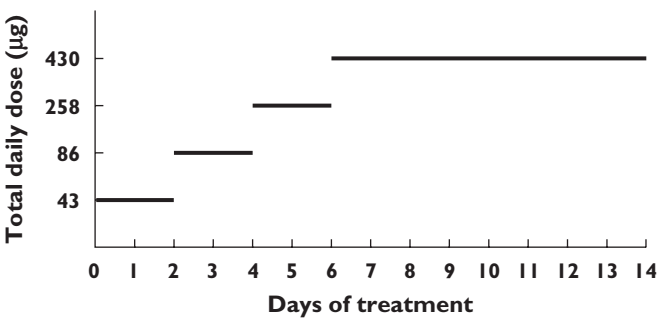

Figure 1

Dose regimen followed during the study. The initial three doses were applied on two consecutive days, whereas the highest dose tested was applied for the remaining 8 days

day^{-1}, days 3 and 4), 86.0 µg (258 µg day^{-1}, days 5 and 6), and 143.3 µg (430 µg day^{-1}, days 7 to 14). Subjects were followed up for an additional 7–10 days after the last eye drop administration in order to assess possible delayed adverse side-effects.

The medical and nursing staff of the Clinical Research Centre of the University Hospital Basel assessed ocular (local) tolerability every day, three times a day before eye drop instillation. Local ocular tolerability was examined for the following solicited symptoms: irritation, redness of the eye, sensation of burning, blurred vision, eye watering. In addition, a slit lamp examination was performed daily. Safety assessments for local tolerability were made daily by the investigator at baseline and at the time of adverse events during the whole duration of the study (14 days), at study end and at follow-up (between 7 and 10 days after end of treatment).

Assessment of clinical tolerability

Assessment of tolerability was obtained through a complete medical history evaluation of past or present cardiovascular, respiratory, gastrointestinal, renal, hepatic, neurological, endocrine, lymphatic, haematological, immunological, dermatological, psychiatric, genitourinary, and surgical history or any other diseases or disorders. Any currently active medication was recorded. Physical examinations was performed and included an examination of general appearance, body weight, head, eyes, ears, nose and throat, neck, skin, cardiovascular system, respiratory system, abdominal system, nervous system. Vital signs [blood pressure and pulse rate (supine after 3 min rest and standing position after 1 min of standing) and body temperature] were recorded. Computerized 12-lead ECG recordings were obtained (rhythm, ventricular rate, PR interval, QRS duration and QT/QTc).

Assessment of biological tolerability was performed at baseline and at end of treatment using clinical laboratory tests. Haematology tests included full blood count including red blood cell count, haemoglobin, haematocrit, packed cell volume, mean corpuscular volume, mean cell haemoglobin, mean cell haemoglobin concentration, white blood cell count and differential white cell count. Coagulation tests included activated partial thromboplastin time and prothrombin time and International Normalized Ratio. Serum biochemistry included sodium, potassium, creatinine, urea, alanine amino transaminase, aspartate amino transaminase, bilirubin, uric acid, total protein, triglycerides, cholesterol, calcium and glucose (fasting). Urinalysis included pH, protein, glucose, ketones, urobilirubin, blood, specific gravity and leucocytes. Screen for drug abuse (cannabis and metabolites, cocaine and metabolites, amphetamines, opiates) and alcohol were performed at the discretion of the investigator. Viral serology included hepatitis B surface antigen and anti-hepatitis C virus and HIV.

Plasma quantification of GS-101

We used the ultrasensitive noncompetitive hybridization method described by Yu and colleagues [19] and able to detect 0.1–1.0 nM of phosphorothioate oligodeoxynucleotides in plasma, in order to evaluate the potential bioavailability of GS-101 in plasma following topical application in the eye. Intra-assay, interassay precision and accuracy were determined by the analysis of quality control samples at low (25 pM), intermediate (100 pM) and high (500 pM) concentration levels. The interindividual variability, evaluated with samples from five subjects, was 9.2% at 500 pM. The calibration curve was done in human plasma between 7.81 and 1000 pM and was fitted with a cubic spline algorithm. The intra-assay mean precision (repeatability), calculated with the three quality control samples, was 10.8%, the interassay mean precision (reproducibility) was 17.0% and the accuracy was comprised in a range of 100 ± 30% for these three quality-control samples. The limit of quantification with acceptable reliability and accuracy (±30% and 70–130%, respectively) was defined at 25 pM and the limit of detection was determined at 20.5 pM. GS-101 was found to be stable in human plasma in the tested assay conditions, after at least three freeze–thaw cycles and after storage for about 2 months at –20°C and –80°C.

Results

Between 24 June 2004 and 23 August 2004, 14 healthy male volunteers aged between 22 and 39 years (mean 29 ± 6) were enrolled in this Phase I clinical evaluation. In the first phase of this study, no signs of ocular intolerance were observed and no adverse reactions were reported in the first three treated subjects. One subject discontinued the study prematurely during the first phase due to family reasons and was not available for the last two ophthalmological observations. However, he did return for the end of study examination, which revealed no signs of intolerance. Based on the results of the first three subjects' initial use of low-dose GS-101, it was decided to continue with the dose escalation phase of the study (Figure 1).

Adverse events were recorded and summarized in Table 1. Ten adverse reactions (seven mild, three moderate) were reported in six patients. Bilateral itching, probably of allergic origin, and a dull feeling of pressure were observed in two of the 12 subjects during the ocular intolerance assessment of the dose escalating period. Mild to moderate itching in the treated eye was reported to the study nurse by two subjects on three occasions as reported during the ocular intolerance examination. Bilateral itching, also in the control eye, was reported in one additional volunteer. Mild to moderate headache was reported twice by one subject. Dull pressure of mild intensity in the treated eye was reported by two subjects as also reported during the ocular intolerance examination. In addition, one

subject presented signs of a common cold and one subject reported muscular pain on the day following the last day of eye drop treatment. All events resolved and were determined to be unrelated to the study treatment. No serious adverse events were reported, and in no case was the study treatment interrupted.

No changes or abnormalities were observed in the ocular examination including visual acuity, slit lamp examination, eye fundus and intraocular pressure measurements performed at screening and after the end of the treatment. No abnormalities were observed between the beginning and end of treatment in the assessment of clinical tolerability. Physical examination, vital signs and 12-lead ECG were all normal. Changes from baseline to end of study in laboratory parameters are shown in Table 2. No abnormality was observed.

In five patients tested, GS-101 was not detectable in the plasma 1.5 h after administration of one drop of the highest concentration (143.3 µg per drop).

Table 1

Summary of adverse events (AEs) recorded during the study

AEs	Number of patients
Itching of both eyes and dull feeling of pressure	2
Itching of both eyes	1
Itching in the treated eye	2
Feeling of dull pressure in the treated eye	2
Headache	1
Muscular pain	1
Common cold	1

Discussion

The phenomenon of angiogenesis is complex and the implications of excessive angiogenesis are huge. IRS-1 involvement in angiogenesis has been demonstrated *in vitro* and in animal experiments, and the ability of GS-101 to inhibit IRS-1 has been validated [17, 18]. Previous *in vitro* investigations have shown that GS-101 inhibits IRS-1 expression in human endothelial cells placed under different angiogenic stimuli [17]. Partial IRS-1 inhibition by GS-101 induced a significant inhibition of both VEGF-A and interleukin-1β transcription in cultured human endothelial cells associated with complete inhibition of capillary-like tube formation [17], an *in vitro* model of angiogenesis. Accordingly, topical administration of GS-101 in the injury-induced corneal neovascularization rat model inhibited *in vivo* angiogenesis [17, 18]. The results of this 'first-in-man'

Table 2

Changes from baseline to the end of the study in laboratory parameters, mean values

Parameter	Unit	BL $n = 12$	End $n = 12$	STD $n = 24$	Change BL to end
Haematology					
Haemoglobin	g dl^{-1}	154	148	9.0	5.58
Haematocrit	fraction	0.43	0.43	0.0	0.01
Erythrocytes	10^{12} l^{-1}	5.0	4.8	0.4	0.19
Leucocytes	10^{9} l^{-1}	5.5	4.9	1.4	0.62
Neutrophils	10^{9} l^{-1}	2.9	2.8	0.7	0.09
Lymphocytes	10^{9} l^{-1}	1.9	1.8	0.4	0.10
Monocytes	10^{9} l^{-1}	0.30	0.32	0.1	0.02
Eosinophils	10^{9} l^{-1}	0.21	0.22	0.2	0.01
Platelets	10^{9} l^{-1}	242	243	40.6	1.42
Clinical chemistry					
ALT	U l^{-1}	21	18	7.4	2.67
AST	U l^{-1}	21	22	5.7	0.67
Bilirubin	µmol l^{-1}	18	18	9.4	0.17
Alkaline phosphatase	U l^{-1}	62	61	19.5	1.67
Albumin	g l^{-1}	43	43	1.8	0.67
Creatinine	µmol l^{-1}	87	84	9.3	3.00
Chloride	mmol l^{-1}	103	105	2.3	1.50
Sodium	mmol l^{-1}	140	139	2.2	1.42
Cholesterol total	mmol l^{-1}	4.8	4.4	1.8	0.43
Potassium	mmol l^{-1}	3.9	3.8	0.3	0.07
Gamma GT	U l^{-1}	21	17	7.9	3.75
Glucose	mmol l^{-1}	4.7	4.6	0.4	0.10
HDL-cholesterol	mmol l^{-1}	1.4	1.4	0.2	0.05
Phosphate	mmol l^{-1}	1.1	1.0	0.2	0.04
Protein total	g l^{-1}	75	74	2.6	1.25
Blood urea	mmol l^{-1}	4.6	4.4	0.6	0.17
Uric acid	µmol l^{-1}	355	340	59.9	15.2

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high-density lipoprotein.

open-label Phase I tolerability study reported here demonstrate that GS-101 is well tolerated in healthy human subjects. A hybridization assay has been established and validated for the detection of GS-101 in human plasma at extremely low levels (20.5 pM); using this assay, GS-101 was not detectable in the plasma following GS-101 administration, strongly suggesting that it is essentially cleared and degraded locally, therefore strongly limiting the possibilities of systemic off-target effects.

Although the functional consequences of inhibiting IRS-1 expression by GS-101 could be investigated further in diseases where excessive angiogenesis is found, orphan indications of corneal graft rejection, retinopathy of prematurity and neovascular glaucoma have been targeted as the first indications for its potential clinical use. In a recent Phase II clinical study of 40 patients with aggressive corneal neovascularization and nonresponsive to conventional therapy, GS-101 was reported to induce highly significant regression of corneal neovascularization compared with the placebo group, where corneal neovascularization progressed in all patients [20].

In conclusion, GS-101 eye drops are safe at the maximum dose used in this study, i.e. 143.3 µg per drop, three times daily. Further investigations in Phase II and III clinical studies are needed for the development of GS-101 as a novel anti-angiogenic therapeutic agent.

Competing interests

None declared.

This study was supported by Gene Signal International SA, Epalinges, Switzerland.

REFERENCES

1 Campochiaro PA. Retinal and choroidal neovascularization. J Cell Physiol 2000; 184: 301–10.

2 Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1995; 1: 27–31.

3 Neumann E, Frithz A. Capillaropathy and capillaroneogenesis in the pathogenesis of rosacea. Int J Dermatol 1998; 37: 263–6.

4 Risau W. Mechanisms of angiogenesis. Nature 1997; 386: 671–4.

5 Liang L, Zhou T, Jiang J, Pierce JH, Gustafson TA, Frank SJ. Insulin receptor substrate-1 enhances growth hormone-induced proliferation. Endocrinology 1999; 140: 1972–83.

6 Senthil D, Ghosh Choudhury G, Bhandari BK, Kasinath BS. The type 2 vascular endothelial growth factor receptor recruits insulin receptor substrate-1 in its signalling pathway. Biochem J 2002; 368: 49–56.

7 Shaw LM. Identification of insulin receptor substrate 1 (IRS-1) and IRS-2 as signaling intermediates in the alpha6beta4 integrin-dependent activation of phosphoinositide 3-OH kinase and promotion of invasion. Mol Cell Biol 2001; 21: 5082–93.

8 Vuori K, Ruoslahti E. Association of insulin receptor substrate-1 with integrins. Science 1994; 266: 1576–8.

9 White MF. IRS proteins and the common path to diabetes. Am J Physiol Endocrinol Metab 2002; 283: E413–22.

10 Delafontaine P, Song YH, Li Y. Expression, regulation, and function of IGF-1, IGF-1R, and IGF-1 binding proteins in blood vessels. Arterioscler Thromb Vasc Biol 2004; 24: 435–44.

11 Miele C, Rochford JJ, Filippa N, Giorgetti-Peraldi S, Van Obberghen E. Insulin and insulin-like growth factor-I induce vascular endothelial growth factor mRNA expression via different signaling pathways. J Biol Chem 2000; 275: 21695–702.

12 White MF. The IRS-signaling system: a network of docking proteins that mediate insulin and cytokine action. Recent Prog Horm Res 1998; 53: 119–38.

13 Jiang ZY, He Z, King BL, Kuroki T, Opland DM, Suzuma K, Suzuma I, Ueki K, Kulkarni RN, Kahn CR, King GL. Characterization of multiple signaling pathways of insulin in the regulation of vascular endothelial growth factor expression in vascular cells and angiogenesis. J Biol Chem 2003; 278: 31964–71.

14 Cursiefen C, Chen L, Dana MR, Streilein JW. Corneal lymphangiogenesis: evidence, mechanisms, and implications for corneal transplant immunology. Cornea 2003; 22: 273–81.

15 Cursiefen C, Rummelt C, Kuchle M. Immunohistochemical localization of vascular endothelial growth factor, transforming growth factor alpha, and transforming growth factor beta1 in human corneas with neovascularization. Cornea 2000; 19: 526–33.

16 Dadgostar H, Waheed N. The evolving role of vascular endothelial growth factor inhibitors in the treatment of neovascular age-related macular degeneration. Eye 2008; 22: 761–7.

17 Al-Mahmood S, Colin S, Farhat N, Thorin E, Steverlynck C, Chemtob S. Potent *in vivo* anti-angiogenic effects of GS-101, an antisense oligonucleotide preventing the expression of insulin receptor substrate-1. J Pharmacol Exp Ther 2009; 329: 496–504.

18 Andrieu-Soler C, Berdugo M, Doat M, Courtois Y, BenEzra D, Behar-Cohen F. Downregulation of IRS-1 expression causes inhibition of corneal angiogenesis. Invest Ophthalmol Vis Sci 2005; 46: 4072–8.

19 Yu RZ, Baker B, Chappell A, Geary RS, Cheung E, Levin AA. Development of an ultrasensitive noncompetitive hybridization-ligation enzyme-linked immunosorbent assay for the determination of phosphorothioate oligodeoxynucleotide in plasma. Anal Biochem 2002; 304: 19–25.

20 Cursiefen C, Bock F, Kruse FE, Borderie V, Wilhelm F, Schirra F, Seitz B, Meller D, Frueh Epstein B, Thiel M. GS-101 eye drops, an antisense oligonucleotide against IRS-1, inhibit and regress corneal neovascularization: interim results of a multicentre double-blind randomized phase II clinical study. Ophthalmology 2009; (in press).