Anti sense and sensibility : renal and skin effects of (antisense) oligonucleotides

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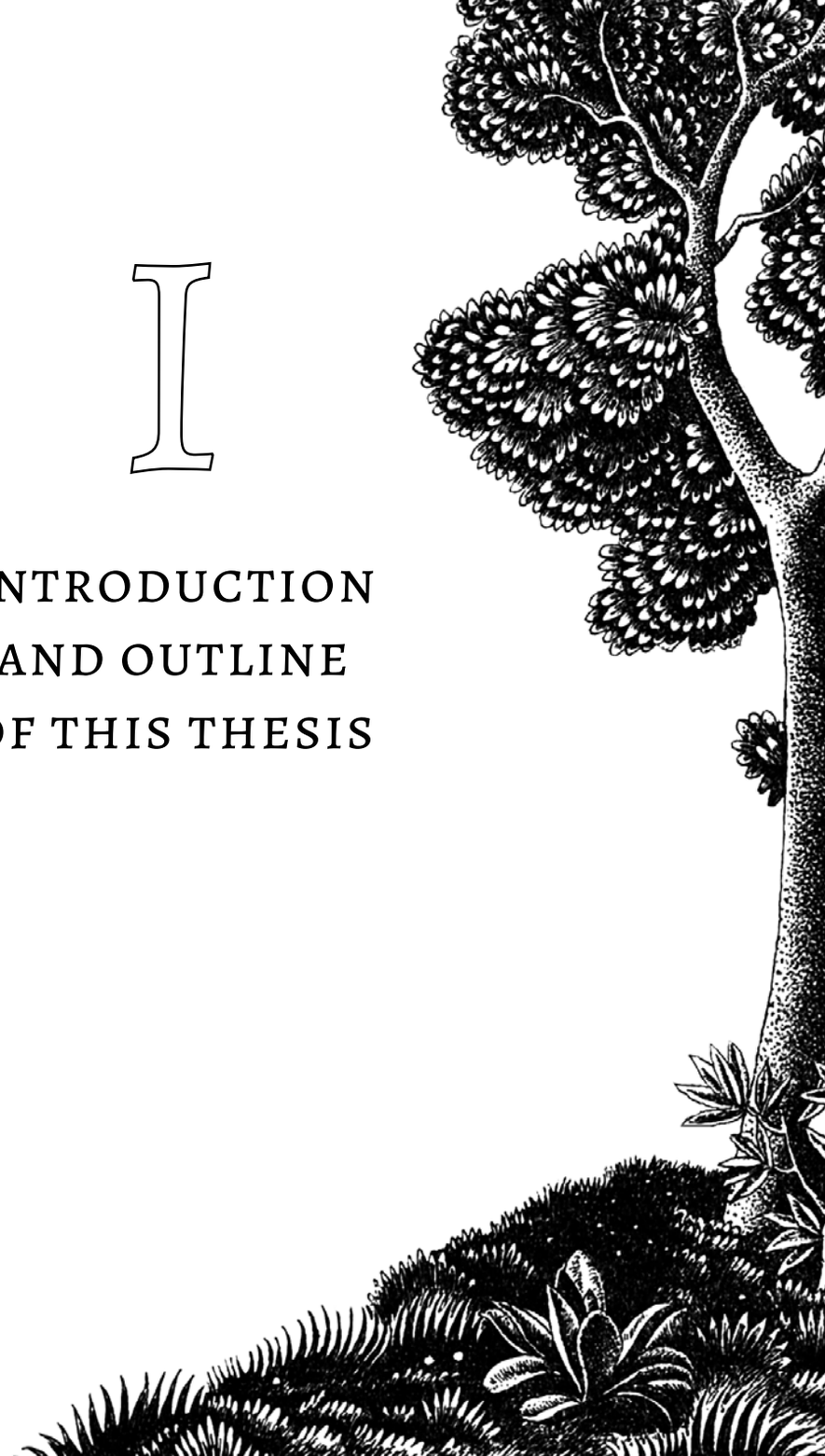
INTRODUCTION AND OUTLINE OF THIS THESIS



Introduction

This thesis describes the clinical investigation of a novel treatment strategy for type 2 diabetes mellitus (T2DM) using an antisense oligonucleotide (AON) to inhibit the SGLT2 receptor. The historical background of both treatment of T2DM and the development of oligonucleotide based therapeutics are covered in this introduction.

TREATMENT OF TYPE 2 DIABETES MELLITUS

About 387 million adults worldwide are living with type 2 diabetes mellitus (T2DM) [1]. The number of affected people is still growing at a fast pace, predicted to result in over 592 million people affected by 2035 [1]. The rise in the prevalence of T2DM in children and adolescents [2;3] contributes largely to this predicted trend. As the metabolic disease state of T2DM interferes with many physiological systems and organ functions, it is associated with a wide range of serious co-morbidities and increased mortality. Optimal treatment of T2DM with tight regulation of serum glucose will be of great importance the coming decades.

Treatment of T2DM began when insulin became available in 1923. Later, in 1955 the first sulfonylureas were developed (table 1). Both insulin and sulfonylureas are associated with burdensome side effects, such as weight gain and hypoglycemia. The next step was the development of metformin, which was introduced in Europe around 1975 and in the United States in 1995 [4]. Currently, metformin still is the preferred drug for initial therapy [5;6]. It is effective and safe and does not affect body weight, which is favorable compared to earlier treatments. Disadvantages include gastrointestinal side effects and its use is contraindicated in patients with severe renal insufficiency. Moreover, monotherapy with metformin is often not sufficient and within 3 years after start of the therapy, 50% of patients need additional drugs to achieve target HbA1c values [7].

Three treatments came available between 1995-1998; thiazolidinediones, α-glucosidase inhibitors and meglitinides. These treatments are less effective compared to metformin, and also have other disadvantages [8]. Thiazolidinediones are associated with weight gain and fluid retention. α-Glucosidase inhibitors and meglitinides need dosing three times daily and are expensive therapies. In 2005 incretin-mimetic compounds reached the market. These drugs have multiple antihyperglycemic actions and induce weight loss, which is promising. As monotherapy however, it is still less effective compared to metformin, insulin and sulfonylureas and long-term safety is not yet established [8].

Elaborating on the mechanisms of action of these drugs is beyond the scope of this thesis. The actions are summarized in table 1 and are more extensively described in the Teaching Resource Centre database [9].

The latest development in the treatment of T2DM are the small molecule SGLT2 inhibitors, which reached the market in 2013. SGLT2 co-transporters are responsible for reabsorption of up to 90% of the glucose filtered by the kidneys [10]. The pharmacological inhibition of SGLT2 co-transporters reduces hyperglycemia by decreasing renal glucose threshold and thereby increasing urinary glucose excretion. This mechanism of action, independent from the amount of circulating insulin or insulin sensitivity is very different from earlier developed therapies, making SGLT2 inhibition potentially useful for patients who are refractory to most other anti-diabetic therapies or as add-on therapy. SGLT2 inhibitors also induce weight loss and a reduction in triglycerides and systolic blood pressure [11;12]. Pharmacological inhibition of the SGLT2 receptor is an accepted concept which led to several registered and approved drugs (dapagliflozin [13] (Forxiga®), canagliflozin [14] (Invokana®) and empagliflozin [15] (Jardiance®)). As opposed to inhibition of the SGLT2 receptor function, antisense oligonucleotides enable inhibition of protein expression, which results in reduction of the abundance of the receptor. This concept led to the preclinical development of ISIS 388626, an antisense oligonucleotide designed to inhibit the SGLT2 receptor via targeting the SGLT2 mRNA. The clinical investigation of this drug candidate is described in this thesis.

OLIGONUCLEOTIDE BASED THERAPEUTICS

Oligonucleotides (ONs) are fragments of 12-24 nucleic acids in a target-specific sequence [16]. ONs have different mechanisms of action. They may be designed to either alter the reading frame by exon-skipping, work directly by inhibition of the targeted protein (antagonism), by binding to the receptor as an agonist or by inhibition of mRNA of the targeted protein using the antisense principle (figure 1). Antisense oligonucleotides (AONs) are complementary to a part of the mRNA of the target protein.

Watson-Crick hybridization of the AON with the mRNA, induces degradation of the mRNA and thereby prohibits translation of the mRNA into the target protein (figure 1).

Oligonucleotides found their origin by the discovery of the DNA structure by Watson and Crick [17] (table 2). It did take some time (25 years) before the first synthesis of short RNA fragments was accomplished [18]. And it lasted another 15 years before proof-of-concept of AONs was established by achieving successful inhibition of gene expression [19]. This led to the development of first generation AONs, of which fomivirsen (for intraocular treatment of CMV retinitis) was the first and only ever approved first generation AON [20]. These first generation compounds were AONs with a phosphorothioate backbone and were relatively stable in the bloodstream. However the duration of action of these compounds was limited and they induced various undesirable, non-specific in vivo side effects, such as immune stimulation and complement activation, which were mainly caused by their interactions with proteins. Around 1999 clinical investigation of 2nd generation AONs started. Many different modifications of the backbone sugar moieties were investigated [21]. The AONs with the most favorable modifications (2'-O-Methyl and 2'-O-Methoxyethyl) became the 2nd generation AONs. These demonstrated greater nuclease resistance and increased hybridization affinity due to more stable duplex formation with the target mRNA [22;23]. 2nd generation AONs are considered to be superior to first generation AONs, therefore the focus in drug development shifted accordingly. 2.5 or 3rd -generation oligonucleotides were developed from 2003 onwards to further improve the nuclease resistance and target affinity [24-26]. Locked nucleic acid (LNA), Peptide nucleic acid (PNA) and Morpholino phosphoroamidates (MF) are the three most commonly used 3rd generation AONs [27]. The clinical benefit of 3rd over 2nd generation AONs has not been established yet. The first and currently only approved 2nd generation compound, is mipomersen. Mipomersen was approved in 2013 by the FDA for the treatment of familial hypercholesterolemia [28].

Administration of all three generations of oligonucleotides, are associated with certain side effects in humans [29]. Intravenous (IV) administration may result in transient prolongation of aPTT [30-32]. This is thought to result from non-specific binding of the AONs with coagulation factors, phospholipids and calcium [33] and is considered to be non-clinically significant. aPTT is not affected when the oligonucleotide is injected subcutaneously probably due to lower plasma concentrations of the drug. Oligonucleotides have pro-inflammatory potential. In clinical studies with subcutaneous injection of the oligonucleotides, injection site responses are commonly observed [29]. IV infusion, as well as subcutaneous injection is associated with constitutional symptoms, such as fever and chills [34-36]. Oligonucleotides also rarely induce hypersensitivity reactions [29]. The pathophysiological and immunological pathways involved in these immune responses is unknown. Complement activation is common in monkeys and is rarely also present in humans [29]. Due to the known distribution pattern of oligonucleotides with accumulation in liver and kidney tissue, these organs are monitored carefully in clinical trials. Many oligonucleotides clearly have no effect on renal performance [29;37], however several other oligonucleotides do induce untoward kidney effects [38-40]. The reason for this discrepancy in unknown. Similarly, increases in liver transaminases have been observed after treatment with some, but not all, oligonucleotides [41-43]. In clinical studies with novel oligonucleotides, these side effects should be monitored closely.

ANTISENSE OLIGONUCLEOTIDE AS SGLT2 INHIBITOR

Inhibition of the SGLT2 transporter using small molecules has proven its efficacy by improving glycemic control in subjects with type 2 diabetes [9;10]. The maximal effect of these compounds is a modest inhibition of only 30-50% of renal glucose reabsorption, whereas complete inhibition of SGLT2 activity could theoretically induce 90% inhibition [10]. This limited effect is not well understood. This could be explained by compensatory increase of SGLT1 activity [44;45], however, another explanation could be that small molecules do not result in sufficient inhibition of the SGLT2 receptor. An alternative approach is to interfere with the synthesis of the receptor by antisense inhibition. ISIS 388626, a 2nd generation 2'-MOE-modified 12-mer oligonucleotide, is a very potent and selective inhibitor of SGLT2 and resulted in ≥ 80% reduction of renal SGLT2 mRNA expression in animal models. Therefore, it was anticipated to be more efficacious than small molecule SGLT2 inhibitors. Down regulation of the SGLT2 protein instead of SGLT2 inhibition with small molecules may mimic hereditary forms of glucosuria, with mutations in the SGLT2 gene [46;47]. Phenotypes resulting from these mutations are not associated

with hypoglycemia nor any other symptoms [46;47]. The specificity and selective renal distribution of ISIS 388626 resulted in potent anti-hyperglycemic effects in several preclinical models [48-50]. No AONs have been developed to date that have their target protein within the kidney. AONs generally have similar tissue distribution patterns with accumulation occurring in liver, kidney, lymph nodes and spleen, therefore the kidney has been suggested as potentially suitable target [51]. ISIS 388626 was designed to specifically distribute to the kidney, due to lower binding to plasma proteins compared to other 20-mer antisense oligonucleotides (88% to 60% bound over a concentration range of 4 to 400 µg/mL, respectively, in rodents, monkeys and dogs, data on file). The higher free fraction of ISIS 388626 in the circulation is subject to greater glomerular filtration and tubular reabsorption, leading to higher kidney concentration. This selective renal distribution was confirmed in animals using radiolabeled ISIS 388626; liver contained measurable levels of ISIS 388626, however these were approximately 5 to 100-fold lower than the levels in the kidneys in both mice and monkeys (data on file). ISIS 388626 did not produce any overt toxicities (specifically, no renal toxicities) in any of the species evaluated, even when SGLT2 mRNA levels were reduced by ≥ 80% [50]. No hypoglycemia was observed in euglycemic or hyperglycemic animals [48-50].

These data supported clinical development of ISIS 388626 for the treatment of T2DM. The objective of the phase 1 study was to provide an initial assessment of the effects of single and multiple subcutaneous doses of 50, 100, 200 and 400 mg ISIS 388626 in healthy subjects in order to select the effective doses for potential future trials in diabetic patients.

Outline of this thesis

ISIS 388626 is an antisense SGLT2 inhibitor, designed to treat Type 2 Diabetes Mellitus by inducing glucosuria. **CHAPTER 2** describes the results of the first-in-human trial with drug candidate ISIS 388626. This trial was halted early due to unexpected effects on renal function. Preclinical experiments revealed no effects on renal function, therefore the findings in this first in human trial were not well understood.

Additional and more extensive preclinical testing of ISIS 388626 with different dose regimens provided more insight into the renal effects. An adapted study design without loading dose was proposed to avoid these untoward effects. The study was restarted (with 50, 100 and 200 mg) and the findings are described in **CHAPTER 3**. Unexpectedly and despite changing the dose regimen, transient creatinine increases still occurred in the first cohort (50 mg). Therefore, renal clearance tests were performed in this study to evaluate the impact of 50 mg ISIS 388626 on GFR and renal plasma flow.

The cohort with the renal clearance tests confirmed the transient nature of the changes and revealed no indications for decreased GFR or renal plasma flow, therefore the trial continued with 100 and 200 mg ISIS 388626 treatment, as described in **CHAPTER 4**. These dose levels were expected to induce the pharmacodynamic effect. Renal effects were monitored closely. ISIS 388626 induced glucosuria, however the effect at the dose levels tested (up to 200 mg) was small. Higher doses of ISIS 388626 further increased the urinary levels of renal markers.

CHAPTER 5 explains why nephrotoxicity often is an important issue in early clinical drug trials. Limitations of the 'classic' renal markers such as serum creatinine and urea are addressed. An overview is provided on promising urinary kidney biomarkers. Finally, recommendations are made regarding the selection of suitable urinary biomarkers to monitor renal function and to detect changes early.

Apart from the unintended renal effects, ISIS 388626 treatment also caused skin reactions on the injection site. These injection site reactions (ISRs) are a commonly observed phenomenon after subcutaneous administered oligonucleotides, however little detailed information is available in the literature. **CHAPTER 6** is a review of all publicly available studies on skin reactions caused by oligonucleotide injection, supported by observations done in four clinical trials performed at the CHDR with four different oligonucleotides, including ISIS 388626. A comprehensive and detailed overview of the incidence, severity, clinical manifestations and pathophysiology of ISRs is provided.

Finally, **CHAPTER 7** combines the findings of the previous chapters, and addresses the risk-benefit assessment of oligonucleotide based therapies in general. Suggestions are done for further research in order to optimize the potential of oligonucleotide based therapies.

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Figure 1 The antisense oligonucleotide is transported across the plasma membrane (1). In the cytoplasm, the single-stranded oligonucleotide enters cell nucleus (2), where it bind to the target mRNA (3). The oligonucleotide/RNA duplex is a substrate for RNase H (4). RNase H cleaves the mRNA strand (5), creating dysfunctional mRNA that is not translated into protein. RNase H is also present in the cytosol, cleavage also occurs in that cellular compartment.

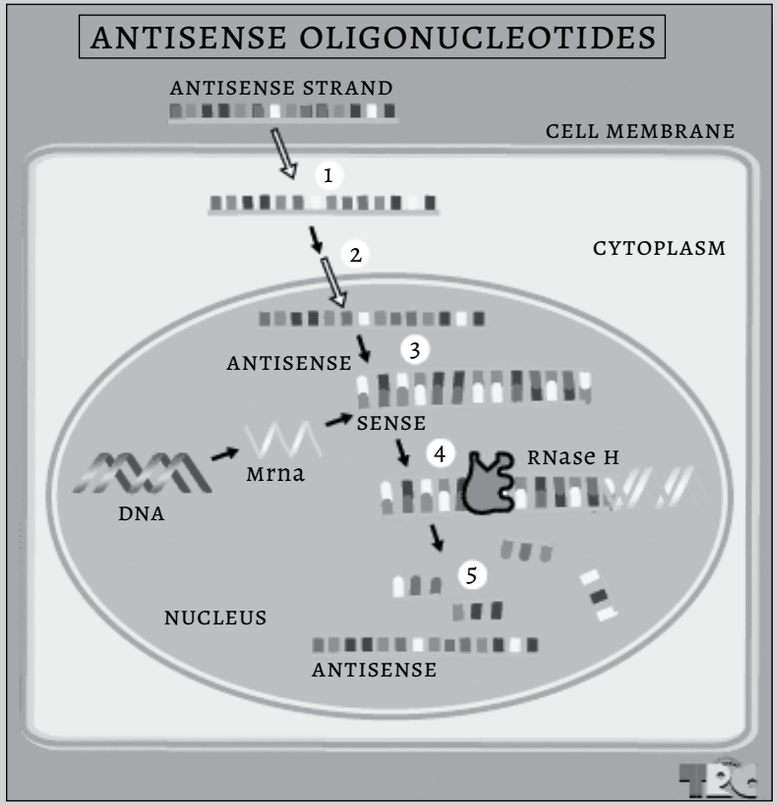

Table 1 Overview of historical development of drugs for the treatment of T2DM (↓ indicates inhibiting effect, ↑ indicates stimulatory effect)

Year	Drug	Actions
1923	Insulin	Hepatic glucose production ↓ Glycogen storage liver muscles ↑ Glucagon ↓
1955	Sulfonylureas	Production insulin β cells ↑
1975	Biguanides available Europe	Hepatic glucose production ↓
1983	2nd generation Sulfonylureas	Production insulin β cells ↑
1995	Biguanides available in the US	Hepatic glucose production ↓
1996	α-Glucosidase inhibitors	Digestion carbohydrates ↓
1997	Thiazolidinediones	Insulin sensitivity liver ↑
1998	Meglitinides	Production insulin β cells ↑
2005	GLP1 analogues	Production insulin β cells ↑ Insulin sensitivity ↑ Glucagon ↓ Gastic emptying, appetite, food intake ↓
2006	DPP4 inhibitors	GLP1 ↑ Production insulin β cells ↑ Insulin sensitivity ↑ Glucagon ↓ Gastic emptying, appetite, food intake ↓
2013	Small molecule SGLT2 inhibitors	Urinary glucose excretion ↑

Table 2 Overview of historical development of oligonucleotide drugs

Year	Drug	Actions
1923	Insulin	Hepatic glucose production ↓ Glycogen storage liver muscles ↑ Glucagon ↓
1955	Sulfonylureas	Production insulin β cells ↑
1975	Biguanides available Europe	Hepatic glucose production ↓
1983	2nd generation Sulfonylureas	Production insulin β cells ↑
1995	Biguanides available in the US	Hepatic glucose production ↓
1996	α-Glucosidase inhibitors	Digestion carbohydrates ↓
1997	Thiazolidinediones	Insulin sensitivity liver ↑
1998	Meglitinides	Production insulin β cells ↑
2005	GLP1 analogues	Production insulin β cells ↑ Insulin sensitivity ↑ Glucagon ↓ Gastic emptying, appetite, food intake ↓
2006	DPP4 inhibitors	GLP1 ↑ Production insulin β cells ↑ Insulin sensitivity ↑ Glucagon ↓ Gastic emptying, appetite, food intake ↓
2013	Small molecule SGLT2 inhibitors	Urinary glucose excretion ↑

Table 3 Overview of generation oligonucleotides and chemical modifications

Generation	Chemical modifications
First generation	Phosphorothiate backbone
Second generation	2-O-Methyl (2-OME) 2′-O-Methoxyethyl (2′-MOE)
Third generation (2.5 or 3rd)	Locked nucleic acid (LNA) Peptide nucleic acid (PNA) Morpholino phosphoroamidates (MF)