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ORIGINAL ARTICLE

Injection site reactions after long-term subcutaneous delivery of drisapersen: a retrospective study

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Abstract

A retrospective study in which we reviewed the hospital files of a subset of 7 patients with Duchenne muscular dystrophy participating in the open-label phase I/II PRO051-02 study in Leuven. The objective of this study was to describe in detail the injection site reactions in these children treated with drisapersen (PRO-051), a 2′-*O*-methyl phosphorothioate RNA antisense oligonucleotide, that induces exon 51 skipping in Duchenne muscular dystrophy. Antisense oligonucleotides, restoring the reading frame by skipping of exons, have become a potential treatment of Duchenne muscular dystrophy and other monogenetic diseases. Erythema followed by hyperpigmentation, fibrosis, and calcification were seen at the injection sites in all children. Ulcerations, which were difficult to heal, occurred in 5 of 7 children. Progression still occurred after switching to intravenous administration of drisapersen or even after stopping therapy. Systemic reactions included a reversible proteinuria and α1-microglobulinuria. Moreover, hypotrichosis was a common feature.

Conclusion: Subcutaneous administration of drisapersen causes severe and progressive injection site effects.

What is known:

- *Antisense oligonucleotides offer the possibility to convert Duchenne muscular dystrophy to the less severe Becker type. This can potentially be achieved by targeting and skipping specific exons of the Duchenne muscular dystrophy gene to restore the disrupted reading frame and to induce the production of a semi functional dystrophin protein.*
- *Drisapersen is such an antisense oligonucleotides which can be administered subcutaneously. Its use has been tested extensively in the escalating dose pilot study (PRO051-02).*

What is new:

- *This report describes the injection site reactions caused by this type of agent in detail which has never been done before. We therefore reviewed the hospital files of 7 patients with Duchenne muscular dystrophy participating in the phase I/II open-label, escalating dose pilot study (PRO051-02) with drisapersen.*
- *Severe side effects starting with erythema, hyperpigmentation, and later fibrosis, calcification, and difficult to treat ulcerations developed in all patients, and these continued to progress even after cessation of drisapersen. We discuss some possible underlying mechanisms. The exact mechanism however is still not known.*

Keywords Duchenne muscular dystrophy · Antisense oligonucleotides · Drisapersen (PRO-051) · Injection site reactions

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Abbreviations

2OMePS	2′-*O*-methyl phosphorothioate
AON	Antisense oligonucleotide
DMD	Duchenne muscular dystrophy
ISR	Injection site reactions
LNA-PS	Locked nucleic acid-phosphorothioate
MOE-PS	Methoxyethyl-phosphorothioate
PS	Phosphorothioate

Introduction

Duchenne muscular dystrophy (DMD) is a rare X-linked recessive muscle disorder primarily affecting skeletal and cardiac muscles and characterized by a progressive loss of function that starts in early childhood. Subjects affected with DMD will lose the ability to walk during their teens, followed by loss of upper limb and cardio-respiratory function ultimately leading to a premature death in their twenties [10]. Affected individuals rarely survive beyond their mid-twenties [11].

The disease is caused by mutations in the dystrophin gene, resulting in a frame shift or premature stop codon. This leads to dystrophin deficiency at the myofiber membrane causing fiber degeneration and progressive replacement by fibro-adipose tissue [9]. Similar mutations, however, with preservation of the reading frame and the production of a shorter semi functional dystrophin protein, cause the much milder phenotype of Becker's muscular dystrophy [5, 9, 10].

A strategy to treat DMD is to correct the mutated reading frame of DMD transcripts by antisense oligonucleotides (AONs), yielding internally truncated but semi functional dystrophins such as those associated with Becker's muscular dystrophy [11, 13]. Drisapersen (PRO-051) is a 2′-*O*-methyl phosphorothioate RNA antisense oligonucleotide that induces exon 51 skipping in DMD [2]. This compound has been investigated in a comprehensive clinical development program.

In this paper, we report the injection site reactions (ISR) observed after long-term subcutaneous (SC) administration in a subset of 7 patients participating in the open-label study PRO051-02 study in Leuven [7, 8]. These ISR seem to be a class adverse effect of PS AONs which are also under investigation to treat other diseases [15].

Material and methods

All 7 children were involved in a dose escalation study followed by a 188-week open-label extension. In the escalation study, each patient received 5 SC injections of 4 possible doses (0.5, 2, 4, or 6 mg/kg). In the open-label study, all patients received weekly SC injections of 6 mg/kg/week. SC injections were first given in abdominal subcutaneous fat; thereafter, they were rotated to the upper arms, buttocks, lower back, and thighs. (ClinicalTrials.gov NCT01910649).

After 188 weeks, intravenous administration was explored until September 2013. IV dosing was resumed in October 2014 until July 2016 when drug development was stopped by the sponsor. The total doses administered per child (SC or IV) are given in Table S1.

All subjects were on a conventional treatment with systemic corticosteroids (daily deflazacort) for at least 6 months prior to inclusion in the study and received Vitamin D supplements.

Results

Adverse events, including ISR (Figs. 1 and 2, Table S1) as well as systemic side effects, occurred in all children.

Erythema was the first sign (Fig. 1a), transient at first but persistent later on. This was followed by hyperpigmentation (Fig. 1b). Induration of lesions started after 8 to 13 months and lesions became frankly sclerotic after 1 to 2 years (Fig. 1c). Severe painful calcifications were eventually seen in all children. These started as fine calcified pinpoint lesions, sometimes with spontaneous transcutaneous elimination. Later, large bone hard plaques occurred; they gave rise to discomfort and were hindering on movement (Fig. 1d, e). They occurred at the abdomen and thighs in all children though less severe and more rarely at the other injection sites. These calcified plaques continued to progress after switching to intravenous treatment and even after treatment stopped. Eventually, they varied in size from 6 to > 20 cm^2. Other ISR that occurred, but not in all patients, were ulceration and lipoatrophy. Painful ulceration, frequently provoked by minor trauma, e.g., scratching or mechanical abrasion, in these indurated, calcified, lesions occurred in 5/7 patients (4 lesions on the abdomen, 1 on the thigh (Fig. 1f)). These ulcers were very slow to heal and healing was only possible after removing all of the calcified tissue. Colonization with *Staphylococcus aureus* was frequent, sometimes provoking cellulitis in the area surrounding the wound. Lipoatrophy was a feature frequently seen at the injection sites of the upper arms.

Treatment of ulcers consisted of preparing the wound bed by removing all non-vital tissue (curettage and in the most severely affected patients surgical removal of complete calcified plaques). Afterwards, hydrogels or alginogels were applied. Despite these approaches, healing was difficult. Cleaning of the wounds was emphasized, sometimes with the use of antiseptics (10 min of Prontosan®) or, if cellulitis was present, oral antibiotics were used. In patient 5 (who had the most recalcitrant ulcers) treatment, in addition to curettage and wound care, consisted of negative pressure therapy. When the wound bed was granulating, keratinocyte grafts were placed.

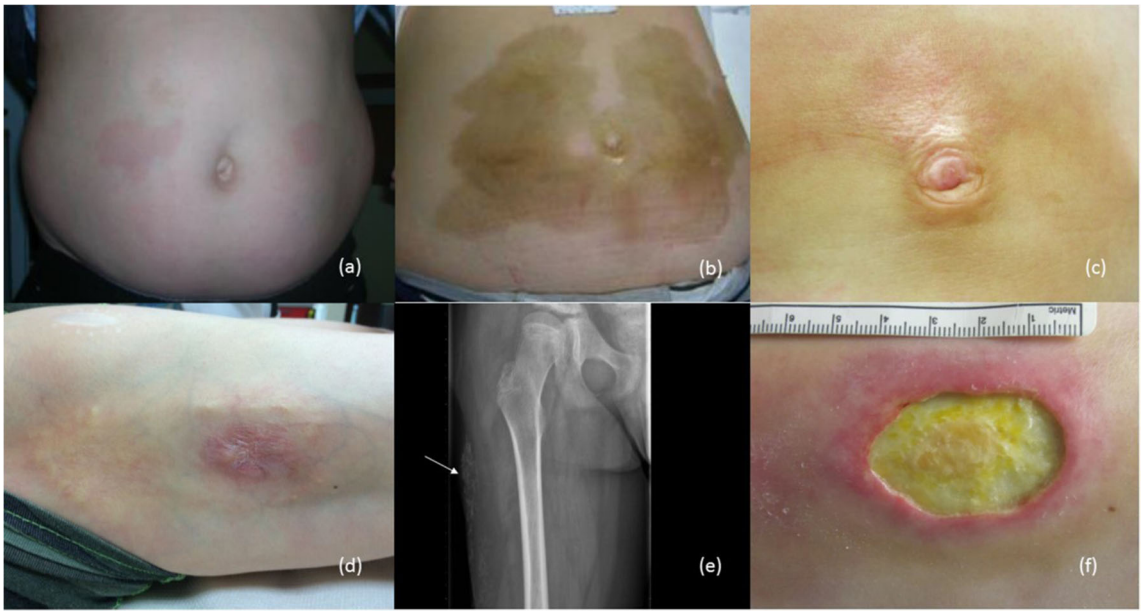

Fig. 1 Clinical features of ISR in chronical order of appearance: (**a**) erythema at injection sites, (**b**) hyperpigmentation, and (**c**) induration (scleroderma-like) frequently starting at the periumbilical skin. (**d**) Calcifications were seen not only at the abdomen, but also at the thighs (**e**) and calcifications were visible on X-ray. (**f**) typical ulceration with stone hard wound bed, which is a calcified plaque. Healing was not possible without removing the plaque

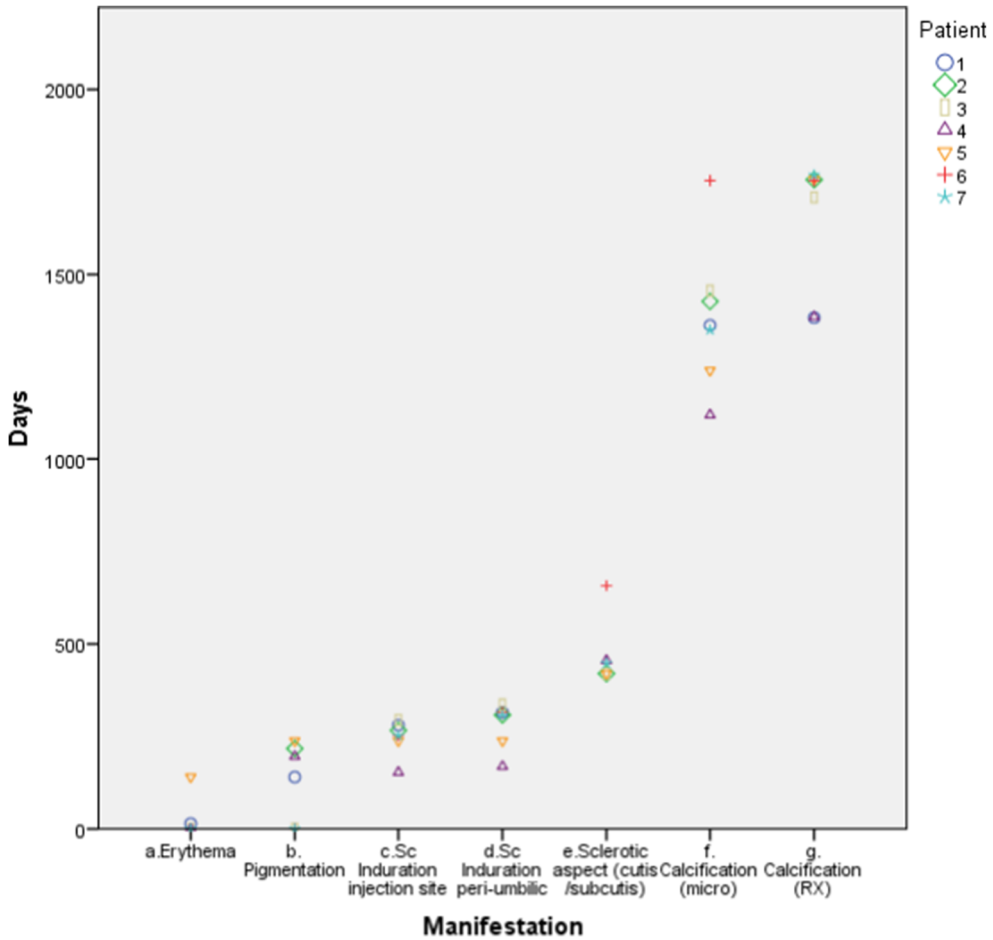

Fig. 2 Graphical display of the time of appearance of the different skin complication after SC injections with drisapersen

Systemic adverse reactions occurred as well; flu-like reactions were seen in one subject, α1-microglobulinuria and low-grade proteinuria in all children. Decreases in thrombocyte count, liver function disturbances, and anemia were more rarely seen. All were reversible during drug holidays. Four of the 7 children developed hypotrichosis mainly of the scalp hair, and in one patient also of the eyelashes and eyebrows.

Histopathology of a fibrotic lesion was performed in one child and showed a normal epidermis with scattered fibroblasts with abundant cytoplasm and large nuclei underneath. In the deep dermis, extensive fibrosis replaced the superficial part of the subcutaneous fatty tissue. Amidst the collagen fibers around high endothelial venules, as well as in the thickened septa of the subcutaneous fatty tissue, nodular aggregates of lymphocytes were present.

Discussion

Therapeutic oligonucleotides are considered as an attractive class of compounds. It is a personalized treatment as the oligonucleotides are designed to correct specific disease-causing mutations [15]. The field in which they can be used is diverse.

Drisapersen is a synthetic 2′-*O*-methyl phosphorothioate RNA (2OMePS) which binds to exon 51 of the DMD gene [3, 4, 14] and induces specific exon-51 skipping, supposedly by causing steric block via hybridization to pre-mRNA thereby interfering with splicing regulators and/or structures and thus altering pre-mRNA splicing [1]. It is able to restore the reading frame for transcripts with different deletions in the exon 45 to 63 region (applying to 13% of DMD patients) and is highly sequence-specific thereby minimizing the risk for off-target effects [14, 17].

Despite clinical efficacy being demonstrated in the PRO051-02 study, all children had adverse effects, including ISR in all. They were progressive, most prominent when injections were only given in abdominal SC fat, and started within weeks after the first injections with erythema and pigment disturbances resulting in fibrosis and calcification after years. ISR are reported in all SC-injected AONs studied to date [15]. The children in the cohort described here however had more severe reactions. The poor prognosis of DMD and clear clinical results stimulated families to go on with treatment despite the side effects. Moreover, the most severe reactions appeared after years of dosing and were progressive even after stopping treatment [6]. When injection sites were rotated to arms and legs, with fewer injections per site, these problems were less prominent although not completely absent, arguing that the cumulative dose of injected oligonucleotide may be important in inducing the ISR. This was already demonstrated in dose finding studies with most other AONs [15]. The time course of events seen in our patients is also compatible with a cumulative effect. The $t_{1/2}$ of PS AONs is typically over 4 weeks [18] (estimated mean 29 days for drisapersen [7]). Recall reaction at the initial injection sites after changing site location has been described with mipomersen, an AON used for hypercholesterolemia [15].

Van Meer et al. [15] suggested a uniform standardized ISR scoring grading system for ISR and the action to take: Grade 0 (no interference), Grade 1 mild (minimal interference), Grade 2 moderate (functional interference), Grade 3 severe and undesirable. They proposed classifications of maximal diameters for the ISR for mild (less than 5 cm), moderate (less than 10), and severe (less than 15 cm or any diameter if associated with systemic reaction or flare-up of previous reactions). Based on our experience, we suggest adapting this by omission of less than 15 cm and change to larger than 10 cm as we have seen

Table 1 ISR scoring grading system for ISR and suggested action to take (adapted from Van Meer et al. [15]). ADL = "Activities of daily living" are defined as bathing, dressing and undressing, feeding self, using the toilet, taking medications, preparing meals, shopping for groceries or clothes, using the telephone, etc.

	0 = none	1 = mild	2 = moderate	3 = severe and undesirable
Injection site reaction	None	Erythema OR tenderness OR itching	In addition to that listed in 1; pain OR swelling OR signs of inflammation	Ulceration or necrosis OR calcification
Maximal diameter ISR	NA	Max 5 cm	Max 10 cm	> 10 cm OR any diameter and systemic reaction OR flare-up previous ISR
Duration of symptoms	≤1 day	2–14 days	2–6 weeks, reversible	Permanent
Sequelae	None	Minimal and tolerated by patient	Hardly tolerated OR wish for treatment by patient	Permanent despite treatment OR no treatment options
Likely impact on next dose	None	Injection site can be used in rotation AND no dose adaptation	Injection site should be avoided in rotation OR change dose regimen	Injection site cannot no longer be used OR discontinuation
ADL limitations	None	Minimal	Functional	Self-care limitations

patches exceeding 20 cm. Moreover, we suggest to add calcification to the severe ISR without limit of extent as this would also lead to the recommendation to stop the SC injections (Table 1).

The pathophysiologic mechanism of these side effects remains elusive. It seems unlikely that off-target effects are produced as the design of PRO051 was highly specific for exon 51 of the DMD gene. Moreover, inflammation of the skin seems to be specific for the subcutaneous delivery of these AONs and is time related. Finally, similar reactions are seen with AONs with completely different sequences. It has indeed been described with all SC-delivered phosphorothioate (PS) AONs (including 2′-*O*-methyl phosphorothioate (2OMePS) RNA, 2′-*O*-methoxyethyl (MOE-PS) RNA, and locked nucleic acid (LNA-PS) RNA) [15].

Association of SC injections in some patients with flu-like symptoms and elevation of CRP levels as well as the nature of the skin responses are arguments that AONs are pro-inflammatory. Although the chemical modifications such as cytosine methylation and 2′-*O*-substitutions on the ribose (as used for DMD therapy) are known to antagonize activation of pathogen-associated molecular pattern-recognition receptors and thus increase tolerance [12], non-substituted PS AONs have been demonstrated to stimulate the innate immune response and are even used as adjuvant to enhance the response to vaccines [18].

Circumventing these ISR seems an important goal for future development of AONs. One way could be chemical changes aimed to reduce pro-inflammatory characteristics. Another way is to change the mode of delivery. The IV route is a possibility but even oral delivery has been tested recently [16]. If ISR are initiated by innate immune reactions, the goal has to be to bypass the cells that initiate the inflammatory response.

Conclusion

We report here on serious ISR provoked by subcutaneous administration of the AON drisapersen in DMD children, starting with erythema and hyperpigmentation followed by induration, calcification, and ulceration. All these ISR were irreversible. Mild systemic reactions were also seen, except for hypotrichosis these were all reversible after stopping treatment.

It is important to unravel the pathogenesis of these side effects especially if it is due to a pro-inflammatory effect by stimulating innate immune reactions.

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Authors' contribution N.H. (leading author), N.G. and MA.M. (senior author) collected and analyzed the data. N.H. and MA.M drafted the manuscript and designed the figures. N.G. contributed to the writing of the manuscript. I.S. analyzed portions of the data and made corrections in the tables of the final version. All authors revised the final version of the article before submitting it.

Compliance with ethical standards

Conflict of interest Nathalie Goemans was principle investigator for PRO051. However, no potential conflicts of interest were reported by the authors.

Informed consent All subjects have given their consent to use their data from the PRO051 study for further research and articles.

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