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Research paper

Development of stable liquid formulations for oligonucleotides

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ABSTRACT

Oligonucleotide-based therapeutics have been implemented as a new therapeutic modality in biotech industry, which offers the opportunity to develop formulation platforms for robust parenteral formulations. The aim of this study was to gain a better understanding of stabilizing/de-stabilizing effects of different formulation parameters on unconjugated and N-acetylgalactosamine (GalNAc) conjugated single stranded oligonucleotides with locked nucleic acid modifications (LNA SSO), as model oligonucleotides. Various buffer systems, pH levels and different excipients were evaluated to optimize conditions for LNA SSO in liquid formulations. LNA SSO were exposed to different temperature conditions, mechanical stress as well as oxidative conditions, and the maximum feasible LNA SSO concentrations regarding handling and processing were determined. Finally, options for terminal sterilization of LNA SSO were evaluated. Results show that the tested LNA SSO were most stable under slightly alkaline conditions. A decrease in viscosity was best accomplished in the presence of spermine and lysine. Heat treatment and gamma irradiation caused high levels of degradation of the LNA SSO. Crucial formulation parameters, as identified in this study, should contribute to a significant increase in future productivity in drug product development for single-stranded oligonucleotides.

1. Introduction

Oligonucleotide-based therapeutics emerge as an additional major drug modality which opens new doors to biotech industry by affecting targets that are considered as “undruggable” by small molecules or protein therapeutics [1]. So far only a few FDA approved oligonucleotides are on the market: Vitravene (fomivirsen sodium; CIBA Vision Corporation/Ionis Pharmaceuticals) since 1998 [2], Macugen (pegaptanib sodium linked to polyethylene glycol; Pfizer/Valeant Pharmaceuticals) since 2004 [3], Kynamro (mipomersen sodium; Kastle Therapeutics/Ionis Pharmaceuticals) since 2013 [4], and Exondys 51 (eteplirsen; Sarepta) [5] and Spinraza (nusinersen; Biogen/Ionis) [6] since 2016. These approvals, as well as the development of a better understanding of the basic biology of oligonucleotides, emerging concepts for improved chemistries, more sophisticated delivery systems and increasing success in the clinics, contribute to a significant growth in the field of oligonucleotide-based therapeutics [7,8]. There are many different subclasses of oligonucleotide based therapeutics including antisense [9], splice-switching oligonucleotides [10], small interfering RNA [11], microRNA [12], aptamers [13] and immunostimulatory oligonucleotides [14,15]. Much research is currently being done on nucleic acid chemistry to improve biostability, structural stability, as well as pharmacological actions of such molecules [16–22]. For parenteral applications, aqueous liquid formulations of oligonucleotide therapeutics are the most economical way to manufacture and the most convenient for the end user [23,24]. However, instability of oligonucleotides is more likely to occur during storage in aqueous solution compared to frozen or lyophilized formulations [25]. Until today it has not been thoroughly investigated, which formulation parameters increase stability of oligonucleotides in aqueous parenteral formulation regarding processing, storage stability and application. A number of external destabilization aspects are linked to binding events to the multiple inherent anionic charges of oligonucleotides [26,27], exposure to UV or visible light during manufacturing, storage and handling [28,29], forces they are exposed to during processing (filtration, tubing, pumping) and administration to the patient (syringeability) [30]. Degradation of oligonucleotides may be caused as a consequence of depurination/ beta-elimination, oxidation, or radical attack [31–34].

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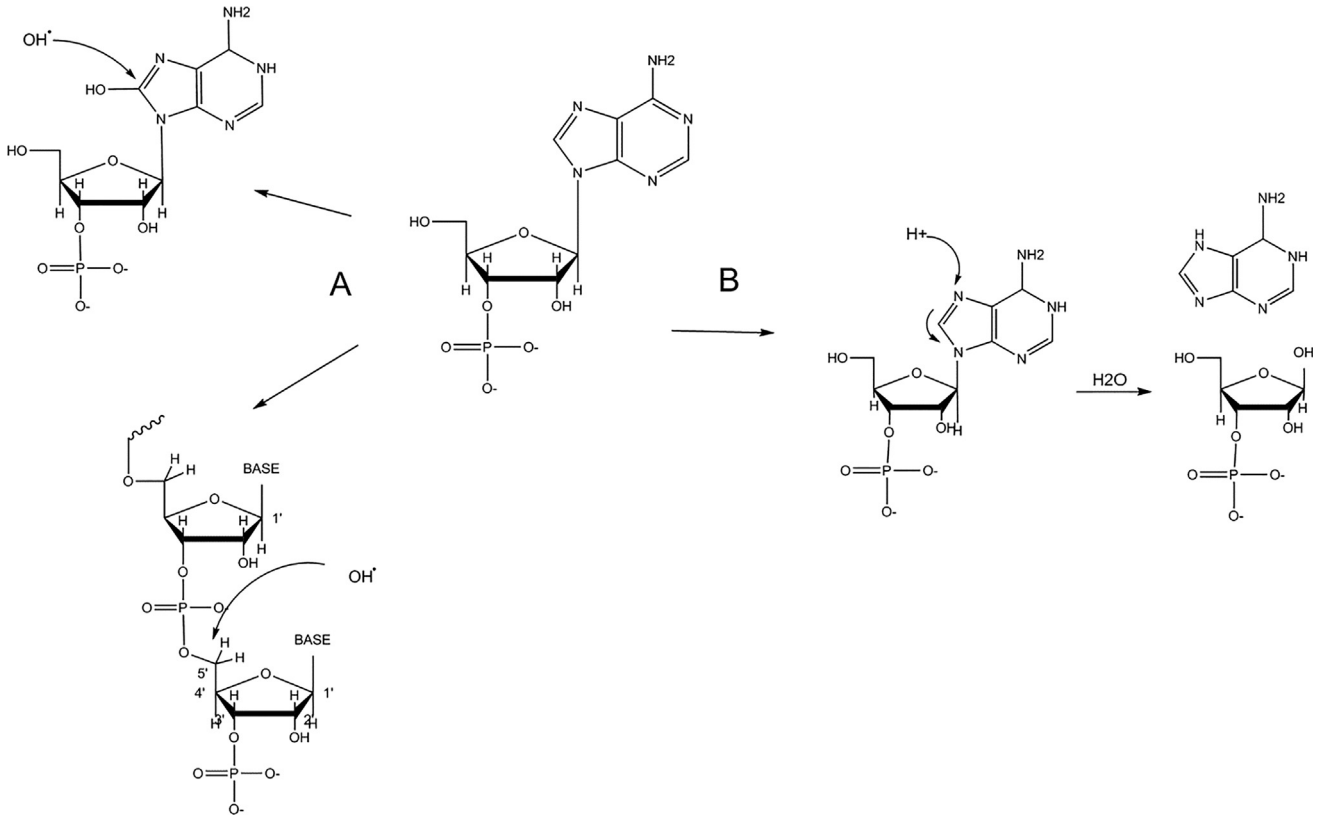

Fig. 1. (A) Free radicals, such as reactive oxygen species, induce oxidation of bases and abstract hydrogens from the C5′ position of ribose leading to strand cleavage by formation of a 3′-phosphate and 5′-aldehyde. (B) Depurination is caused by protonation at the N3-nitrogen of purines leading to the weakening and subsequent cleavage of the glycosidic C1′N9 bond.

Therefore, various aspects need to be considered, including excipients as stabilizers, compatibility of ingredients and the development of appropriate analytical methods. In the manufacture of sterile products, as it is the case for parenteral formulations, the use of terminal sterilization, such as moist heat sterilization, gamma irradiation or sterile filtration should be applied. Where this is not feasible without damaging the product, aseptic processing has to be implemented in the manufacture to achieve sterility. In this case proper scientific explanation and justification needs to be provided [35,36].

In this article the basic behavior of unconjugated or N-acetylgalactosamine (GalNAc) conjugated single stranded oligonucleotides with locked nucleic acid modifications (LNA SSO), as model oligonucleotides, their instabilities, and stabilization in liquid formulation will be discussed. The aim was to evaluate various parameters such as pH, buffer systems and viscosity decreasing agents to protect the LNA SSO in liquid formulation from potential hydrolysis, strand breakage, and oxidation (Fig. 1) and to overcome manufacturing, stability, analytical and delivery challenges in the development of high-concentration LNA SSO formulations. For the development of the manufacturing process, consideration was given to the possibility of terminal sterilization by heat and gamma irradiation of the finished vials, containing the LNA SSO formulations.

2. Materials and methods

2.1. Materials

Unconjugated (5′-TS GS GS cS aS aS gS cS aS tS cS cS TS GS TS a -3′) and conjugated (5′-GalNAc-C6O cO aO AS GS mCS gS aS aS gS tS gS cS aS cS AS mCS G -3′) LNA/DNA gapmers for this study were supplied by F. Hoffmann-La Roche Ltd. (Basel, Switzerland) as lyophilized powders, where GalNAc is a trivalent N-acetylgalactosamine cluster, C6 is an amino linker, upper case letters denote beta-D-oxy LNA, lower case letters denote DNA, “mc/ mC” denotes a 5-methylcytosine DNA/LNA, “S” denotes phosphorothiate internucleoside linkages, and “o” denotes phosphodiester internucleoside linkages. Water from a Millipore ion-exchange unit (Milli-Q) was used for preparing the liquid formulations and dilutions. For the preparation of buffers, citric acid monohydrate and sodium phosphate monohydrate were purchased from Univar Benelux (Brussels, Belgium), trisodium citrate dihydrate from Jungbunzlauer Suisse AG (Basel, Switzerland), disodium hydrogen phosphate and tris(hydroxymethyl)aminomethane from Merck (Darmstadt, Germany), tris(hydroxymethyl)aminomethane hydrochloride from Sigma-Aldrich (St. Louis, USA), histidine and histidine hydrochloride from Ajinomoto (Tokyo, Japan), sodium hydrogen carbonate from Fisher Chemical (Loughborough, UK) and sodium carbonate anhydrous from Burdick & Jackson (Honeywell Burdick & Jackson, Morristown, USA). As viscosity decreasing agents sodium chloride from Fisher Chemicals (Loughborough, UK), calcium chloride and ethanol from Merck (Darmstadt, Germany), magnesium chloride from Sigma (St. Louis, USA), L-lysine from Serva Electrophoresis GmbH (Heidelberg, Germany) and spermine from Acros Organics (Morris Plains, NJ, USA) were applied.

2.2. Analytical methods

2.2.1. LNA SSO content determination

The content of unconjugated and conjugated LNA SSO was determined by absorption spectroscopy using a Lambda 35 UV spectrophotometer (Perkin Elmer, Waltham, USA) and measuring at a wavelength of 260 nm. Samples were diluted with water before measurements to ensure A_{260} reading values between 0.5 and 1 optical density. The moisture content of the lyophilized drug substances of unconjugated and conjugated LNA SSO, intended for formulation, was