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Biodistribution and metabolism of immunostimulatory oligodeoxynucleotide CPG 7909 in mouse and rat tissues following subcutaneous administration

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Abstract

To evaluate pharmacokinetics (PK) and biodistribution, CPG 7909, a 24-mer immunostimulatory fully phosphorothioated oligodeoxynucleotide (PS-ODN), was administered by subcutaneous injection at 2, 5 and 12.5 mg/kg to mice and at 9 mg/kg to rats. Parent compound and metabolites were isolated from plasma and tissues and quantified by capillary gel electrophoresis with UV detection (CGE-UV) and molecular masses were determined by matrix-assisted-laser-desorption-ionization time of flight detection (MALDI-TOF). An established method for PS-ODN isolation from plasma and tissue was modified to prevent oxidation of the phosphorothioate bonds during the extraction process, significantly increasing sensitivity in the subsequent MALDI-TOF analysis. Concentrations of CPG 7909 and metabolites were highest at the injection site (>600 mg/kg at 4 h). Maximal concentrations in local (draining) lymph nodes (LLN), kidney and liver were 10–15% of that at the injection site. The highest total amount of PS-ODN (percentage of administered dose) was found in the liver (32% at 4 h), followed closely by the injection site (23% at 4 h). Only very low levels of CPG 7909 and metabolites were found in plasma and only during the first hours. Metabolites identified by MALDI-TOF were similar for both species and all analyzed tissues, although the relative amounts of the different metabolites varied with tissue and over time. Degradation of CPG 7909 in vivo occurred predominantly by 3′exonucleases with additional cleavage by endonucleases.

Keywords: Oligonucleotide; Metabolites; Pharmacokinetics; Capillary gel electrophoresis; MALDI-TOF; Solid phase extraction

1. Introduction

CPG 7909, also known as CpG 2006, is a 24 mer PS-ODN containing three human-optimized immunostimulatory 6 mer CpG motifs and made with a fully phosphorothioate backbone to render it more nuclease resistant [1,2]. CPG 7909 is being developed as a novel first generation drug candidate for immune therapy in cancer indications [3] and is currently in Phase II human clinical trials in non-small cell lung cancer, melanoma and cutaneous T cell lymphoma. CPG 7909 is also being tested as an adjuvant for infectious disease and cancer vaccines [4–6]. Doses in the oncology clinical trials range from 0.08 to 0.64 mg/kg, while adjuvant doses tested range from 0.125 to 1 mg (approximately 0.002–0.01 mg/kg).

PK and tissue distribution of PS-ODN have been described after intravenous, intradermal, intraperitoneal, subcutaneous and intrapulmonary administration [7–21] in odents [10,22–25], primates [12,26–29] and humans

Abbreviations: ODN, oligodeoxynucleotide; PS-ODN, fully phosphorothioated oligodeoxynucleotide; PK, pharmacokinetics; IV, intravenous; SC, subcutaneous; CGE-UV/LIF, capillary gel electrophoresis with UV or laser induced fluorescence detection; MALDI-TOF, matrix-assisted-laser-desorption-ionization time of flight detection; SPE, solid phase extraction; HPLC/ESI-MS, high-performance-liquid-chromatography/electro-spray-ionization-mass-spectrometry; LLN, local (draining) lymph nodes; DTT, 1,4-dithio-DL-threitol

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[30–34]. The PK of various PS-ODN have been reported to be dose dependent, sequence independent and equivalent on a dose per body weight basis across non-human and human species, and it is generally accepted that plasma PK in humans can be extrapolated from other species [23,33]. However, a direct species comparison [28] reported that after IV administration, plasma clearance and distribution half-life were more rapid, and drug concentrations in the major organs of deposition (liver, kidney and spleen) were much lower in mice than monkeys. In plasma, approximately 91–99% of PS-ODN is protein bound with the major protein species being albumin and α2-macroglobulin [35,10].

Although PS-ODN are more resistant to degradation by nucleases relative to oligonucleotides made with an unmodified phosphodiester backbone, degradation does still occur. In general, it is reported that 3′exonucleases are the major degradation pathway [36,37,23,38]. Degradation at early times appears to be rapid with a high percentage of metabolites being detected in the plasma as early as 10 min, however, degradation seems to progress more slowly at later times [19]. Explanations for this include the inhibition of nucleases [14] and/or the distribution of Rp and Sp phosphorothioate diastereoisomeres, which have significantly different nuclease resistance [39,40,41]. Degraded PS-ODN are eliminated primarily by urinary excretion [29,42]. Reports from various clinical studies appear to obtain different pharmacokinetic profiles, however this is likely due in large part to the different analytical techniques used; some methods measure only parent compound, whereas others detect both parent and chain-shortened metabolites. Furthermore, there have been very few studies that have attempted to evaluate metabolite profiles of PS-ODN including identification of metabolites in solid organs. The reported techniques for these studies include CGE-UV/LIF, MALDI-TOF [43], HPLC/ESI-MS [44,45], CGE and ion-pair-reversed-phase-HPLC followed by ESI-MS [46,47] and CGE-UV/ESI [19].

While detection of only full-length PS-ODN may be sufficient for antisense drugs, where virtually all metabolites are expected to be inactive, this is not the case for CPG 7909. Since CPG 7909 contains several hexanucleotide CpG motifs, some of its metabolites also show immunomodulatory properties (Vollmer, unpublished results). Therefore, it is of great interest to identify and quantify the main metabolites of CPG 7909 in the major organs of ODN distribution. Herein we have examined rat and mouse liver, kidney, local lymph nodes and spleen tissues after subcutaneous (SC) administration of CPG 7909 and identified the major metabolites present at various times. For this we have used several techniques including CGE-UV and MALDI-TOF. We also describe a modified method for SPE for PS-ODN that avoids oxidation at the phosphorothioate bonds, a problem that heretofore caused problems for interpreting mass-spectroscopy data of isolated metabolites, as had been observed by us and others [19,43].

2. Methods

2.1. Test article

CPG 7909 is a 24 mer full phosphorothioate oligodeoxynucleotide with the sequence 5′-TCG TCG TTT TGT CGT TTT GTC GTT-3′. The internal control (IS) is a 31 mer full phosphorothioate oligodeoxynucleotide with the sequence 5′-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT T-3′. The CPG 7909 was manufactured by Avecia Biotechnology and the IS was manufactured at Coley Pharmaceutical GmbH.

2.2. Test system

Male and female Sprague–Dawley rats (∼200 g) and female Balb/c mice (∼20 g) were obtained from Charles River. Rats were housed in same sex pairs and mice in groups of up to five mice per cage. During the test period, animals were fed a standard laboratory diet and kept under controlled conditions. All studies were conducted in the Animal Care Facility of Coley Pharmaceutical Group. All animal experiments were subject to approval by the Coley Canada Animal Care Committee, under the guidelines and requirements of the Canadian Council on Animal Care (CCAC).

2.3. Test article administration

All animals were weighed immediately prior to dosing. All animals received the indicated dose of CPG 7909 in a total volume of either 2.5 ml/kg (rats) or 0.1 ml (mice). All administrations were given under light isofluorane anaesthetic. Administration was by single SC bolus injection.

2.4. Sample collection

Plasma: blood samples (∼0.5 ml) were collected after 10 and 30 min, and 1, 2, 4 and 8 h via the tail artery after anaesthetization with isofluorane, and centrifuged at 13,000 rpm for 10 min to enable plasma collection. Plasma samples were stored at $\leq -15\ ^{\circ}C$ until processed for analysis. Tissue samples: selected tissues (injection site, liver, kidney, spleen, LNN) were removed from euthanised animals (isofluorane inhaled anaesthetic followed by exsanguination via abdominal aorta) after 4 h, 48 h or 7 days, individually weighed, and then stored at $\leq -15\ ^{\circ}C$ until processed for analysis.

2.5. Tissue and plasma extraction

ODN were extracted from tissues pieces (∼100 mg) or plasma (100 μl). Typically, 1.25 μg of internal standard