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Pharmacokinetics of Bcl-2 antisense oligonucleotide (G3139) combined with doxorubicin in SCID mice bearing human breast cancer solid tumor xenografts

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Abstract Purpose: To evaluate the pharmacokinetic (PK) properties of Bcl-2 antisense oligodeoxynucleotide G3139 when combined with the anthracycline anticancer drug doxorubicin (DOX) in a model of MDA435/LCC6 human breast cancer in severely compromised immunodeficient (SCID) mice. Methods: An orthotopic model of MDA435/LCC6 solid breast tumors was developed by bilateral implantation of passaged cells in female SCID-RAG2 mice. The G3139 plasma profile was compared for two common routes of administration (i.v. or i.p.) in single and multiple dose treatment regimens of 5 mg/kg G3139 alone or with simultaneous DOX (5 mg/kg) administration. At selected times, plasma and major organs were assayed for [3H]G3139 using scintillation counting and DOX determined using HPLC. The molecular integrity of G3139 was analyzed using SDS-PAGE. The PKs of G3139 and DOX were evaluated using a two-compartment model. Results: G3139 administered i.v. at 5 mg/kg revealed a biexponential plasma concentration-time curve with a Cmax of 99.9 µg/ml and elimination half-lives of 0.03 h and 9.8 h, respectively, which resulted in an area under the concentration-time curve (AUC) of 17.4 µg·h/ml. G3139 administered i.p. showed a plasma absorption, distribution and elimination profile typical of this route of administration, characterized by half-lives of 0.03 h, 0.2 h and 8.9 h, respectively and a Cmax of 8.6 µg/ml. Based on AUC comparisons, the bioavailability of G3139 injected i.p. was 84% compared to i.v. administration. Subtle changes were observed in G3139 PKs after three prior i.p. doses of G3139. Specifically, a six-fold slower absorption rate, lower Cmax (6.9 µg/ml), increased Tmax (0.2 h), and an AUC of 17.4 µg·h/ml were observed, consistent with concentrations approaching saturation levels in tissue sites to which G3139 distributes. Coadministration of DOX had significant effects on the PK properties of G3139, manifested by an increased Cmax (11.2 µg/ml), higher AUC (19.7 µg·h/ml), and ninefold lower plasma clearance for single-dose G3139 administration. G3139 in plasma remained largely intact (< 17% degraded in plasma over 4 h), and increased plasma protein association occurred as a function of time. G3139 was detected in both healthy and tumor tissue after i.v. and i.p. administration. The highest tissue levels of G3139 were observed in the kidneys (40 µg/g), and low levels (< 2 µg/g) were detected in lung, heart and muscle. The rate of accumulation of G3139 in organs was dependent upon G3139 levels in plasma and the presence of coadministered DOX. Significant accumulation of G3139 was observed in solid tumors, with peak levels of approximately 5 µg G3139/g tumor, and approximately a two- to threefold tumor/muscle AUC ratio. The kinetics of G3139 accumulation in tumor tissue increased with increasing circulating G3139 concentration. The tissue distribution properties of DOX were also altered in the presence of coadministered G3139: in the presence of G3139, tumor exposure to DOX increased two- to threefold without alteration in plasma DOX PKs. Conclusions: These findings indicate that drug-drug interactions between G3139 and DOX are modest and favorable in that elevated tumor DOX levels are achieved without compromising G3139 tumor uptake or significantly altering plasma drug concentrations.

Keywords Anti-sense · Bcl-2 · Doxorubicin · Pharmacokinetics · Breast cancer
Abbreviations  AS: antisense  ·  AUC: area under the concentration-time curve  ·  Cmax: maximum concentration  ·  CL: clearance  ·  DOX: doxorubicin  ·  i.p.: intraperitoneal  ·  i.v.: intravenous  ·  K01/K10: respective absorption and elimination rate constants  ·  K01 half-life/ K10 half-life: respectively absorption and elimination half lives  ·  ODN: oligonucleotide  ·  PK: pharmacokinetics  ·  SCID: severely combined immunodeficient  ·  t1/2: distribution half life  ·  t1/2β: elimination half life  ·  Tmax: time to Cmax  ·  V: volume of distribution

Introduction

Overexpression of the oncogene product Bel-2 is associated with chemoresistance in a variety of cancers, the mechanism involving the inhibition of programmed cell death or apoptosis [1, 2, 3, 4]. Recently, antisense oligonucleotides (AS ODNs) to Bel-2 have been developed as a novel strategy to modulate Bel-2 levels in tumor cells via specifically hybridizing to complementarity regions of the mRNA coding Bel-2 [5]. Downregulation of Bel-2 is believed to occur by direct AS ODN mRNA inhibition and/or degradation of these duplexes by RNAse H resulting in an inhibition of Bel-2 protein translation [6, 7]. Specific properties of AS ODNs, i.e., ODN sequence, chemical modification of the ODN sequence, stability, RNA binding affinity, RNase H activity, and cellular uptake, determine the effectiveness of the ODN as a therapeutic agent [8].

Recently, a phosphorothioate AS sequence directed against the open reading frame of the Bel-2 RNA (G3139; Genta, Berkeley Heights, N.J.) has been shown to be effective in a variety of tumor models in vitro and in vivo and is currently in clinical development [9, 10, 11]. Since many cytotoxic drugs are believed to act by inducing apoptosis, concurrent Bel-2 AS ODN and drug treatment presents an effective strategy resulting from possible AS ODN-drug synergism [12, 13, 14, 15, 16, 17, 18]. Prior to combining AS ODNs with chemotherapeutics, understanding the in vivo PKs of each drug is imperative to identify potential drug interactions that may result in adverse or favorable biological responses. PK studies allow predictions of drug bioavailability, drug exposure to target tissues, and identification of organs of potential toxicity. Few studies have examined the PK properties and tumor delivery of G3139 [9, 19]. Furthermore, there are no published reports on the effects of multiple treatments or coadministered chemotherapeutics on the PK properties of G3139. Given the high DNA-binding avidity of certain anticancer drugs, such as doxorubicin (DOX), possibilities exist for DOX-G3139 interactions both in the circulation and in tissues, and these could affect the bioavailability of both agents.

In this study, we evaluated the PK properties of G3139 in MDA435/LCC6 breast cancer tumor-bearing SCID mice to define the PK properties of G3139 in the presence and absence of DOX, and in order to evaluate possible G3139-drug PK interactions and help elucidate the relationship between therapeutic responses and the concentrations of G3139 in plasma and solid tumors.

Materials and methods

AS ODN and drugs

The phosphorothioate ODN (18-mer), G3139, with a sequence complementary for the first six codons of the open reading frame of Bel-2 mRNA (5'-tct ecc gtc ggc cat-3', molecular weight 5684.58 Da) was used as the AS ODN, and was a gift from Gentra (Berkeley Heights, N.J.). [3H]G3139 was prepared by inserting a non-exchangeable [3H] at the 5' position of the thymidine of G3139 (Trilink Biotechnologies, San Diego, Calif.). Doxorubicin (DOX) was from Faulding (Vaudreuil, Quebec). Acetonitrile, acetone, propan-2-ol, and ammonium formate were of either analytical or HPLC grade.

Cell lines, mice and tumor models

The human breast cancer cell line MDA435/LCC6 was obtained from Dr. R. Clarke, Georgetown University [20]. Female SCID-RAG2 mice (4-6 weeks of age, 18–22 g) were obtained from the BC Cancer Agency Joint Animal Facility breeding colony and kept in an aseptic environment. MDA435/LCC6 cells were routinely maintained by serial passages of ascites i.p. in SCID-RAG2 mice. An orthotopic tumor model of MDA435/LCC6 cells in RAG2 mice was established by bilateral implantation of 2×106 in vivo-passaged MDA435/LCC6 cells into the mammary fat pad. All animal protocols were approved by the B.C. Cancer Agency Animal Welfare Committee.

PKs and tissue distribution

PK experiments with [3H]G3139 were conducted in female RAG2 mice bearing MDA435/LCC6 tumors (0.1–0.15 g). The PKs of G3139 (5 mg/kg) were compared after a single bolus injection either i.v. or i.p.. In addition, the PK properties of G3139 (5 mg/kg) were examined after three consecutive i.p. doses given on days 1–4. The PKs of G3139 were also studied in the presence and absence of DOX (5 mg/kg) given i.v. 1 h before the G3139 injection. At selected times after G3139 treatment, mice (three per group) were euthanized by CO2 asphyxiation over 6 h. Blood was collected via cardiac puncture and placed into EDTA-coated microtainer tubes. Plasma was isolated from whole blood by centrifugation at 500 g for 10 min. Major organs (i.e., liver, spleen, lung, heart, kidney), muscle and solid tumor were dissected, rinsed in PBS, dried, and weighed into glass tubes. A 20% homogenate of liver in distilled water was prepared using a Polytron homogenizer (Kinematica, Switzerland). [3H]G3139 was determined in plasma and tumor/ tissue homogenates using Solvable (Packard, Missasuga, Ont.), a tissue solubilizer, (50°C overnight) and treatment with a cocktail of 200 mcmd EDTA, 30% H2O2 and 10 N HCl for 1 h at room temperature. The amount of radioactivity in samples was determined using scintillation counting (TRI-CARB Model 1900, Packard Instrumentation, Meriden, Ct.).

HPLC analysis of DOX

DOX in plasma and tissues was determined using a previously described HPLC method [21, 22]. Briefly, DOX was extracted from plasma or tissue homogenates with acetonitrile and reconstituted in mobile phase consisting of 16 mM ammonium formate (pH 3.5)/ aceton/isoopropanol (75:20:5). Samples were run using isocratic elution on a Waters 2690 HPLC with a built-in autosampler.