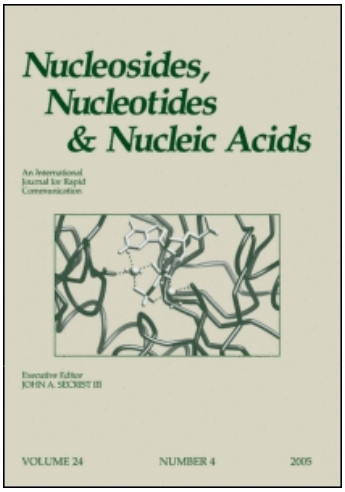

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Oligonucleotide Pharmacology and Formulation: G3139 Anti-BCL2 Phosphorothioate In Stealth® Liposomes and Gel Implants

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OLIGONUCLEOTIDE PHARMACOLOGY AND FORMULATION: G3139 ANTI-BCL2 PHOSPHOROTHIOATE IN STEALTH® LIPOSOMES AND GEL IMPLANTS

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ABSTRACT: G3139, an 18mer phosphorothioate, down regulates BCL2 and is efficacious in human xenograft tumor models. Studies of pharmacokinetics, toxicology, and influence of formulations on the biological properties of G3139 in mice are described. Bioavailability by s.c. and i.p. saline formulation injections are similar, about 60%.

INTRODUCTION

The BCL2 protein inhibits apoptosis, programmed cell death, and is a key target for treatment of many important cancers. G3139, an 18mer phosphorothioate (PS) DNA analogue[1], was developed from earlier antisense oligonucleotides shown to down regulate BCL2[2,3]. It is efficacious in treatment of human xenograft tumor models of melanoma, colon, breast, and other cancers when administered as a saline solution by s.c. infusion for 14 days. Thus the pharmacokinetics (PK), toxicology and potential improvement by alternative formulations have been studied.

METHODS AND MATERIALS

G3139 and ^{35}S-G3139 were prepared with overall purity of 95% or higher by standard methods using standard phosphoramidite chemistry with Beaucage sulfurization. ^{35}S-G3139 was prepared by replacing the final sulfurization step with $^{35}S_8$. All materials were reagent grade. Pharmacokinetic and toxicity studies were performed using standard procedures in BALB/c mice of approximately 20 g, or in cynomolgus monkeys. Plasma

and tissue levels based on total radioactivity and intact G3139 were determined by HPLC[4]. Necropsy and histopathology were performed by a certified pathologist. Sterically stabilized liposome[5] and agarose gel formulations were prepared by standard procedures to be described elsewhere.

RESULTS AND DISCUSSION

Plasma and selected tissue levels over a 24 hr period were determined for G3139 administered as a saline solution at 5 mg/kg. G3139 is rapidly cleared from the blood with multiexponential kinetics; the first half-life is about 5 min and the terminal distribution half-life is about 11 hr, shown in FIG. 1. The bioavailability of G3139 by subcutaneous and intraperitoneal injections is about 60%, regardless of route. As would be predicted from an 11 hr terminal distribution rate and high bioavailability, steady state plasma levels are attained in three days with s.c. infusion, reaching 1 μg/mL with a dose of 5 mg/kg/day. Tissue distribution occurs rapidly with most tissues studied showing significant exposure with the one exception being brain. FIG. 2 shows the distribution of radioactivity into tissues after 24 hr by i.v. injection and s.c. infusion.

14 day s.c. infusion dose ranging studies (up to 100 mg/kg/day) of G3139 were performed. An MTD of 25 mg/kg/day based on survival was determined, but this dose resulted in significant organ toxicities, with liver and heart necrosis appearing responsible for acute dose limiting toxicity. At 15 mg/kg/day only moderate organ toxicities were observed, and those effects disappeared with a 6 week recovery period. Non-dose limiting (sub-acute) toxicity consisted primarily of hyperproliferation of lymphoid cell derived tissues. In particular, a dose dependent b-cell proliferation leading to splenomegaly was observed. Intermittent local inflammation was observed at the site of infusion, but only occurred in the recovery period. Splenomegaly was used as an indicator of the principal toxicity in the recovery studies as well as for the evaluation of biological activity of G3139 when administered as liposome formulations. In cynomolgus monkeys, the MTD was not reached at the highest dose tested, 10 mg/kg/day. Only slight sub-acute toxicities, kidney pigmentation and inflammation at the site of infusion, were observed and generally only at the highest dose.

Alternative routes, schedules, and/or formulations that are more desirable than continuous subcutaneous infusion are of interest. To this end, G3139 has been

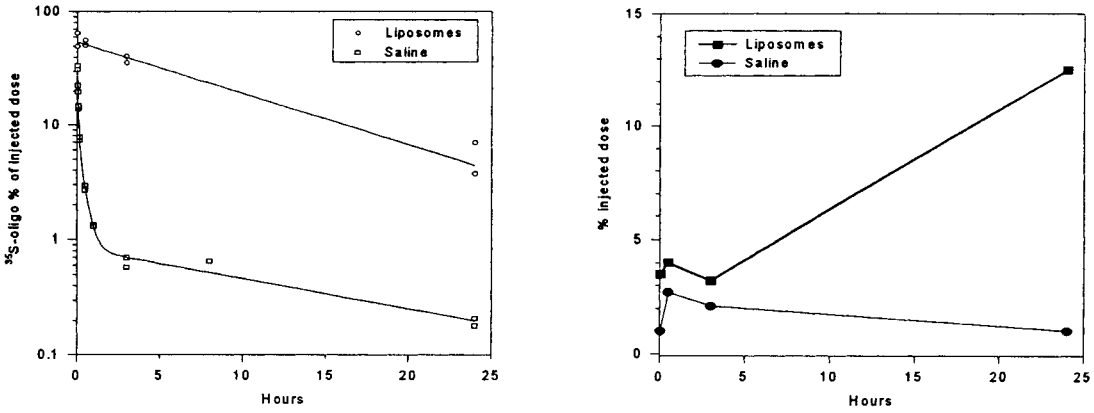

FIG. 1. Pharmacokinetics of G3139 as a saline solution or encapsulated in sterically stabilized liposomes. Left: Plasma clearance following i.v. injection. Right: B16 tumor uptake.

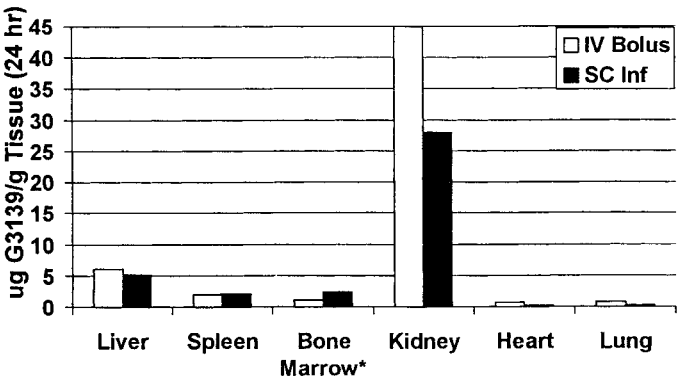

FIG. 2. Tissue distribution of G3139 24 hr after i.v. injection or starting s.c. infusion (*Bone marrow measurements were μg/g protein).

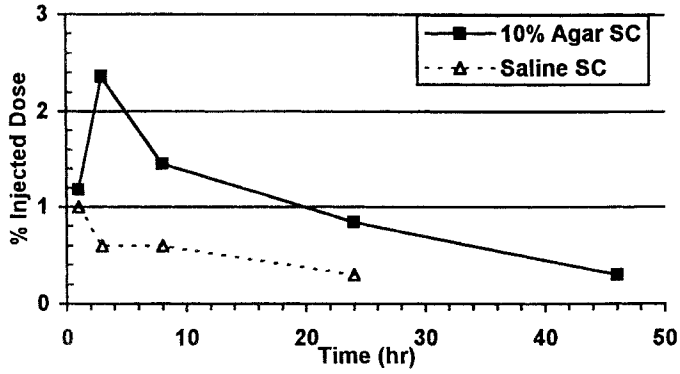

FIG. 3. Plasma levels of G3139 following s.c. saline injection, or s.c. implantation of 10% agarose gel formulation.

encapsulated in sterically stabilized liposomes which have been shown to be long circulating and localize in tumors as well as other sites of pathology. The result is that the clearance and distribution of G3139 is determined by the liposome; plasma clearance is compared with saline solution in FIG. 1. A ten or twenty-fold increase in tumor uptake is achieved, shown in FIG. 1. In addition, polymer gel implants can provide prolonged release of G3139 into the subcutaneous compartment, as determined by observation of prolonged plasma levels, shown in FIG. 3.

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