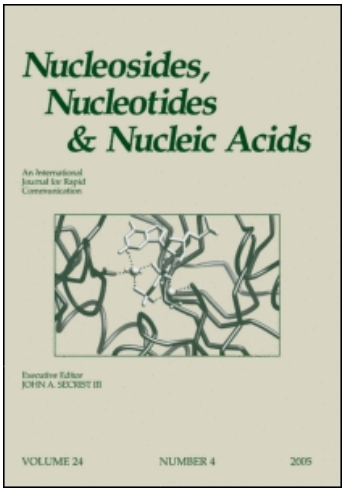

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PHARMACOLOGIC DISPOSITION OF A PHOSPHOROTHIOATE OLIGODEOXYNUCLEOTIDE IN MICE

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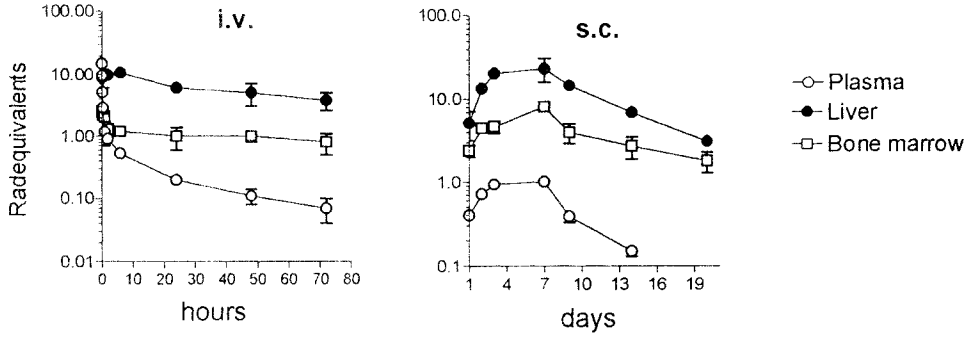
Abstract. G3139, an 18mer phosphorothioate (^{35}S-labeled), was administered to mice by single i.v. bolus or continuous s.c. infusion. The latter schedule resulted in reduced liver metabolism and urinary excretion together with greater tissue accumulation, in particular to the liver, kidney and bone marrow, probably reflecting the dose received.

The t(14;18) chromosomal translocation, in which the *BCL-2* gene is juxtaposed to the immunoglobulin heavy chain gene, results in deregulation and overexpression of the BCL-2 protein. This abnormality, found in most follicular and some high grade lymphomas, may protect cells from apoptosis thus increasing the tumorigenic potential and chemoresistance (1,2). G3139, an antisense fully thioated 18mer oligodeoxynucleotide (5'-TCTCCCAGCGTGCGCCAT-3'), targeted to the first 6 codons of the open reading frame of *BCL-2* has been shown to have antitumor efficacy against a human lymphoma (DoHH2) transplanted into SCID mice. The optimal schedule for tumor eradication was 5mg/Kg/day, given by continuous subcutaneous (s.c.) infusion, over a period of 3 weeks. In these experiments, decreased *BCL-2* expression was observed within the lymphoma (3). In this present study we have examined the pharmacokinetics and metabolism of ^{35}S-G3139 (radiolabeled at the 5'-penultimate base) when administered by either intravenous (i.v.) bolus or continuous s.c. infusion.

Female BALBC mice weighing 20g(±1.2g) were injected i.v. with 100μg(1.92μCi) of ^{35}S-G3139, anesthetized at various time points up to 72 hours and blood and tissues collected for analyses (n=5 animals per time point). Alternatively, primed micro-osmotic minipumps were implanted s.c. dorsally into anesthetized mice to deliver 106μg(2μCi) of ^{35}S-G3139 per day for 7 days after which they were removed. Blood and tissues were collected up to 20 days post implantation (n=3 animals per time point). 3 animals from each of the above groups were placed in metabolism cages for 3 days and urine and feces collected. For radioactivity measurements, tissues and feces were digested in soluene prior to the addition of scintillant, whereas plasma, plasma ultrafiltrate and urine were added directly to scintillant. Following incubation with proteinase K, G3139 and closely related metabolites were phenol extracted from plasma



Radequivalents represent the amount of G3139 which would result in the measured radioactivity (μg/ml plasma, μg/g liver and μg/g protein bone marrow). Error bars = ± SEM.

FIG.1 Radioactivity in radequivalents folowing G3139 administration.

and tissue homogenates and separated by ion-exchange HPLC on a Waters Gen-pak-Fax column using a LiCl linear gradient in LiOH in the presence of 20% acetonitrile.

Following i.v. bolus, most of the plasma radioactivity was protein bound (98% at 5 min) as measured by ultrafiltration through 10,000 MW exclusion membranes. Tissue to plasma ratios were 87 for kidney, 17 for liver, 5 for spleen, 2.5 for heart and lung , and 3.5 for gut. Although levels were detected in the bone marrow over the 72h period of study (FIG. 1), none was detected in the brain. The plasma decay of G3139, determined by HPLC, fitted a 3 compartment model with α, β and γ half-lives of 5 min, 37 min and 11 h respectively. 33% of the administered radioactivity was excreted in the urine in the first 24h and 56% over the 3 days whereas 38% of the radioactivity was recovered in the feces after 3 days (TABLE 1). Only traces of G3139 were detected in the urine.

Following continuous s.c. infusion of ^{35}S-G3139 for 7 days, plasma steady state levels were reached by day 3 (FIG. 1) when half of the radioactivity was protein bound and 65-90% was associated with parent compound over the time period studied. Tissue to plasma ratios were similar to those observed with i.v. administration but tissue accumulation was greater, particularly in the bone marrow. At steady state, the plasma concentration of G3139 approached 2μM and the half-life of elimination was 22h. 7% of the radioactivity was excreted in the urine (feces 3%) during the first 24h and 13% (feces 7%) of the cumulative dose over 3 days (TABLE 1). Less than 3% of parent compound could be detected in the urine which suggests that it is the degradation products which are mainly excreted.

G3139 was metabolized to at least 3 different species; 2 metabolites eluted earlier than G3139 on the HPLC column and the third metabolite after G3139, indicative of a possible addition reaction with the molecule. While the first 2 metabolites were observed in the plasma following i.v. administration, the third metabolite was not detected. This metabolite was never more than 4% of the total radioactivity in the liver

TABLE 1. Cumulative excretion as % of administered radioactivity.

	i.v.		s.c.	
	urine	feces	urine	feces
Day 1	33	17	7	3
Day 2	49	27	9	4
Day 3	56	38	13	7

TABLE 2. Radequivalents (μg/g) following ^{35}S-G3139 administration.

	i.v.		s.c.	
days	liver	kidney	liver	kidney
1	6.1 (40)	45 (82)	5.2 (69)	28 (82)
2	5.1 (60)	21 (82)	13.6 (81)	88 (80)
3	3.8 (90)	13 (39)	20.6 (69)	74 (46)
7	-	-	23.6 (77)	102 (57)
14	-	-	7.0 (63)	7.5 (42)

Figures in parentheses represent G3139 as % of total radioactivity injected onto the HPLC column.

following either route of administration, whereas metabolites 1 and 2 represented up to 79% and 17% of the total radioactivity respectively at various time points.

Following administration by either route, radioactivity concentrated in the liver and kidneys over the time periods studied (FIG.1, TABLE 2). In the kidneys, most of the radioactivity was associated with parent compound during the first 48h. Degradation products were formed mainly by the liver and metabolism was more extensive in the i.v. bolus schedule compared with s.c. infusion. At 24h, when mice had received equivalent doses of drug by both schedules, more G3139 accumulated in the liver (3.6μg/g s.c., 2.4μg/g i.v.) and less in the kidneys (23μg/g s.c, 37μg/g i.v.) with s.c. infusion compared with i.v. bolus. At later time points with the s.c. infusion, accumulation was noted in all tissues and G3139 was still detectable 7 days after removal of the micro- osmotic minipumps.

In summary, we have examined the tissue distribution, metabolism and excretion of ^{35}S-G3139, an 18mer phosphorothioate oligodeoxynucleotide targeted to *BCL-2*, using 2 different routes of administration, single i.v. bolus and continuous s.c. infusion for 7 days. Following both routes of administration, radioactivity was widely distributed and present in all tissues studied except the brain. Radioactivity was excreted in both urine and feces. The highest levels of G3139 were measured in the liver and kidneys. The liver appeared to be the major site of degradation of G3139 which was metabolized

to at least 3 different species. Continuous s.c. infusion resulted in greater tissue accumulation of parent compound, particularly the bone marrow, probably reflecting the larger dose received. In addition, there was less metabolism and excretion compared with single i.v. bolus.

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