

Research Article

S110, a 5-Aza-2'-Deoxycytidine-Containing Dinucleotide, Is an Effective DNA Methylation Inhibitor *In vivo* and Can Reduce Tumor GrowthJody C. Chuang¹, Steven L. Warner⁴, David Vollmer⁴, Hariprasad Vankayalapati⁴, Sanjeev Redkar⁵, David J. Bearss⁴, Xiangning Qiu^{1,6}, Christine B. Yoo¹, and Peter A. Jones^{1,2,3}**Abstract**

Methylation of CpG islands in promoter regions is often associated with gene silencing and aberrant DNA methylation occurs in most cancers, leading to the silencing of some tumor suppressor genes. Reversal of this abnormal hypermethylation by DNA methylation inhibitors is effective in reactivating methylation-silenced tumor suppressor genes both *in vitro* and *in vivo*. Several DNA methylation inhibitors have been well studied; the most potent among them is 5-aza-2'-deoxycytidine (5-Aza-CdR), which can induce myelosuppression in patients. S110 is a dinucleotide consisting of 5-Aza-CdR followed by a deoxyguanosine, which we previously showed to be effective *in vitro* as a DNA methylation inhibitor while being less prone to deamination by cytidine deaminase, making it a promising alternative to 5-Aza-CdR. Here, we show that S110 is better tolerated than 5-Aza-CdR in mice and is as effective *in vivo* in inducing *p16* expression, reducing DNA methylation at the *p16* promoter region, and retarding tumor growth in human xenograft. We also show that S110 is effective by both i.p. and s.c. deliveries. S110 therefore is a promising new agent that acts similarly to 5-Aza-CdR and has better stability and less toxicity. *Mol Cancer Ther*; 9(5); 1443–50. ©2010 AACR.

Introduction

DNA methylation is used by mammalian cells in maintaining a normal expression pattern; it is involved in the regulation of imprinted gene expression and X-chromosome inactivation, among others (1–3). Methylation of CpG islands in promoter regions is often associated with gene silencing and aberrant DNA methylation occurs in most cancers, leading to the silencing of some tumor suppressor genes (4, 5). Reversal of this abnormal hypermethylation by DNA methylation inhibitors is effective in reactivating methylation-silenced tumor suppressor genes both *in vitro* and *in vivo* (6).

DNA methylation inhibitors can be further divided into two groups—nucleoside analogues and nonnucleoside analogues—and most have been well studied for their mechanisms of actions and clinical potentials (6). Nucleoside analogues are converted into nucleotides

and are incorporated into the DNA and there they can trap DNMTs by forming covalent complexes (7–9). 5-Azacytidine (5-Aza-CR) and 5-Aza-CdR are two well-known DNA methylation inhibitors and have been approved by the Food and Drug Administration for the treatment of myelodysplastic syndrome (6, 10, 11). Unlike traditional chemotherapy agents, DNA methylation inhibitors do not induce immediate cell death at their optimal dosage, although cytotoxicity can occur at high concentrations. Cells need to be proliferating for the effective incorporation of drugs into the DNA and reactivate methylation-silenced tumor suppressor genes that in turn make the cells more responsive to apoptotic or cell cycle-regulating signals (6). Given their potential in clinical applications, much effort has been invested to develop more stable forms of these known DNA methylation inhibitors that can be effectively delivered to cancer cells.

Since Laird et al. (12) showed that 5-Aza-CdR was effective in reducing the occurrence of intestinal adenomas in *Apc^{Min/+}* mice, there has been many animal studies that examine the actions of epigenetic drugs. Zebularine, another promising DNA methylation inhibitor, is effective at reducing tumor growth *in vivo* (13, 14). Karam et al. (15) reported that HDAC inhibitor FK228 can inhibit transitional cell carcinoma xenograft growth with minimal undesirable side effects. Moreover, 5-Aza-CdR and zebularine have been shown to decrease vessel formation, a necessary step for tumor formation, in mouse tumor models (16). Many studies have examined the

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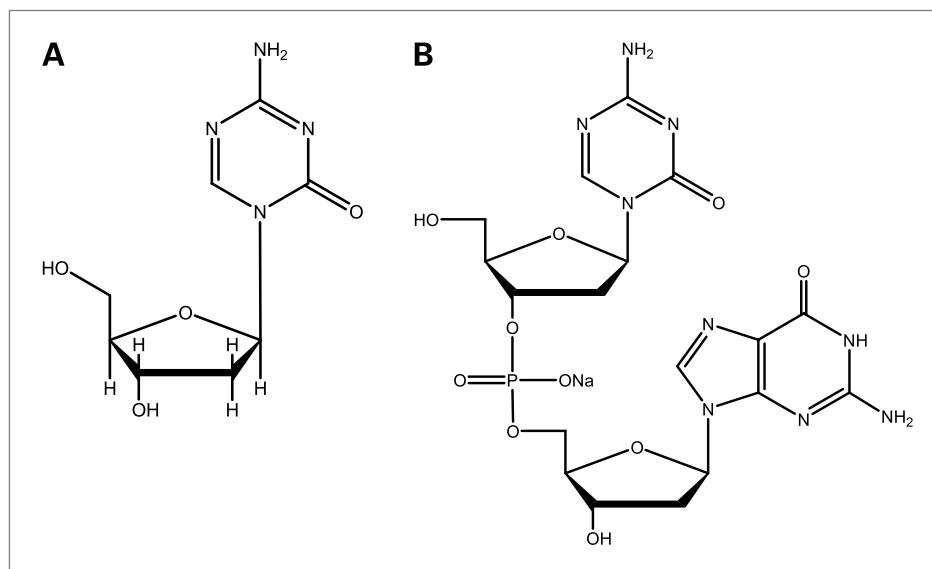


Figure 1. Chemical structures of 5-Aza-CdR (A) and S110 (B).

combinatorial effects of different epigenetic drugs in mouse tumor models (17–19).

We examined the *in vivo* effect of the dinucleotide S110, which consists of 5-Aza-CdR followed by a deoxyguanosine. S110 is effective *in vitro* in inducing *p16* expression and is more stable than 5-Aza-CdR due to the decreased deamination by cytidine deaminase (20). We now show that S110 is better tolerated than 5-Aza-CdR in tumor-free mice and is as effective *in vivo* in inducing *p16* expression and reducing DNA methylation at the *p16* promoter region. We show that S110 is effective at retarding tumor growth in a xenograft model and we also show that the effect can be achieved by both *i.p.* and *s.c.* deliveries. S110 therefore serves as a promising new agent that acts similarly to 5-Aza-CdR and has better stability and less toxicity.

Materials and Methods

In vivo drug tolerability study. Nontumor-bearing athymic *nu/nu* mice (Charles River) were divided into six treatment groups with six animals per group. Treatments of S110 and 5-Aza-CdR were prepared in PBS and administered *i.v.* through tail vein injections. Doses and dosing schedules were designed so that after 7 days each group received molar equivalents of either S110 or 5-Aza-CdR. Animals were treated on the following schedules for 3 weeks: group 1 received 36.6 mg/kg S110 once weekly (Monday) and group 2 was administered 15 mg/kg 5-Aza-CdR once weekly. Group 3 was dosed with 18.3 mg/kg S110 twice weekly (Tuesday and Thursday) and group 4 received 7.5 mg/kg 5-Aza-CdR twice weekly. Finally, groups 5 and 6 received 12.2 and 5.0 mg/kg of S110 and 5-Aza-CdR, respectively, administered thrice weekly (Monday, Wednesday, and

Friday). Tolerability was grossly evaluated by body weight measurements and morbidity. Body weight measurements were recorded twice weekly.

In vivo xenograft drug efficacy studies with *i.p.* delivery. The EJ6 human bladder cancer cell was used for this study and experiments were done similarly to previously described (13). EJ6 cells (5×10^5 /injection) suspended in PBS were inoculated *s.c.* into the right and left back (along the midaxillary lines) of 4- to 6-week-old female BALB/c athymic nude-Foxn1nu mice (Harlan). Mice were randomly divided into three groups. After 2 to 3 weeks and after macroscopic tumors (50–200 mm³) had formed, treatments were initiated. Tumors were measured with calipers, and tumor volumes (TV) were calculated with the following formula: $TV = LD^2/2$ (in which *L* is the longest diameter and *D* is the shortest diameter). The fold differences in tumor growth among the various mice groups were calculated using relative TVs (RTV), which are calculated as follows: $RTV = TV_n/TV_0$, in which TV_n is the TV in mm³ at a given day *n* and TV_0 is the TV in mm³ at day 0 (initial treatment). Mice were weighed at the beginning and end of treatment to determine toxicity. The percent weight change for each mouse was calculated with the following formula: $[(W_6 - W_0)/W_0] \times 100\%$ (in which W_n is the mouse weight on day_{*n*}).

5-Aza-CdR was used as the positive control and 0.45% PBS was used as the negative control. PBS, 5-Aza-CdR (dose of 5 mg/kg in PBS), and S110 (dose of 10 mg/kg in PBS) were administered daily by *i.p.* injection over a period of 6 days.

All mice were sacrificed 24 hours after the last treatment. At this time, tumors were removed and each tumor was divided into two separate portions. One portion was immediately homogenized in Trizol reagent (Invitrogen) for RNA extraction and the other portion

was immediately frozen in liquid nitrogen for DNA extraction later. Genomic DNA and RNA would be used for analysis of the methylation status of *p16* promoter by methylation-specific single nucleotide extension (Ms-SNuPE) and of gene expression by real-time reverse transcription-PCR (RT-PCR), respectively. All experimental protocols were approved by the Institutional Animal Care and Use Committee, in compliance with the Guide for the Care and Use of Laboratory Animals, University of Southern California.

In vivo xenograft drug efficacy studies with s.c. delivery. Athymic *nu/nu* mice (Charles River) were inoculated s.c. in the right hind flank with 1×10^7 EJ6 bladder cancer cells. After tumors reached 0.5 cm in diameter, the animals were stratified into three groups with eight animals per group to begin treatments. Doses and dosing schedules were designed so that each group received molar equivalents of either S110 or 5-Aza-CdR. The agents were administered s.c. once weekly at a dose of 12.2 mg/kg for S110 and 5.0 mg/kg for 5-Aza-CdR for 3 weeks. The study included an appropriate PBS control group. Tumor sizes by caliper and body weight measurements were taken twice weekly to monitor tumor growth inhibition and tolerability.

Nucleic acid extraction. RNA was extracted by first homogenizing tumor samples in Trizol reagent (Invitrogen) and then following the manufacturer's instructions. Genomic DNA was extracted by dissolving each tumor sample in 500 μ L of lysis buffer [100 mmol/L Tris-HCl (pH 8.5), 5 mmol/L EDTA (pH 8.0), 0.2% SDS, 400 mmol/L NaCl, 100 μ g/mL proteinase K, and 10 μ g/mL RNase] overnight at 55°C and then followed by phenol-chloroform extraction (21, 22).

Quantitative real-time RT-PCR. Total RNA was extracted from cells with the Invitrogen Trizol reagent. Reverse transcription (RT) was done with M-MLV reverse transcriptase and random hexamers from Promega. We performed quantitative real-time RT-PCR analysis as previously described (23) with the DNA Engine Opticon System (MJ Research). The primers used are listed below:

p16 sense, 5'-AGC CTT CGG CTG ACT GGC TGG-3'; *p16* antisense, 5'-CTG CCC ATC ATC ATG ACC TGG A-3'; and *p16* fluorogenic probe, 5'-TGG ATC GGC CTC CGA CCG TAA CT-3'. The real-time RT-PCR conditions for both genes were as follows: 95°C for 9 minutes, followed by 45 cycles of denaturation at 95°C for 15 seconds and annealing at 60°C for 1 minute.

Quantitative DNA methylation analysis by Ms-SNuPE. Two micrograms of each genomic DNA sample was converted with sodium bisulfite as previously described (24) and each region of interest was amplified by PCR. The PCR conditions for *p16* were as follows: 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 1 minute, annealing at 62°C for 1 minute, and extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. The bisulfite-specific PCR primer sequences are as follows: *p16* sense 5'-GTA GGT GGG GAG GAG TTT AGT T-3' and *p16* antisense 5'-TCT AAT AAC CAA CCA ACC CCT CCT-3'. The Ms-SNuPE conditions for *p16* were as follows: 95°C for 2 minutes, 50°C for 2 minutes, and 72°C for 1 minute. The *p16* SNuPE primers are as follows: 5'-TTT TAG GGG TGT TAT ATT-3', 5'-TTT TTT TGT TTG GAA AGA TAT-3', and 5'-TTT GAG GGA TAG GGT-3'. The PCR amplicons were extracted with the Qiagen Gel Extraction kit and Ms-SNuPE analysis was done to examine the methylation level changes as previously described (25).

Results

S110 is better tolerated than 5-Aza-CdR in vivo in tumor-free nude mice. Although 5-Aza-CdR is an effective DNA methylation inhibitor both *in vitro* and *in vivo*, it suffers from the drawback of having toxicity such as myelosuppression in patients (26). S110 is a dinucleotide consisting of 5-Aza-CdR followed by a deoxyguanosine (Fig. 1) and has been previously shown by Yoo et al. (20) to be effective *in vitro* as a DNA methylation inhibitor and comparable with 5-Aza-CdR in decreasing DNA methylation, reactivating *p16* expression, depleting

Table 1. Three week tolerability study of S-110 and 5-Aza-CdR with varying schedules

Group	n	Treatment	Dose	Route	Schedule
1	6	S-110	36.6 mg/kg	IV	3 \times Q7D
2	6	5-Aza-CdR	15 mg/kg	IV	3 \times Q7D
3	6	S-110	18.3 mg/kg	IV	3 \times BIW
4	6	5-Aza-CdR	7.5 mg/kg	IV	3 \times BIW
5	6	S-110	12.2 mg/kg	IV	3 \times TIW
6	6	5-Aza-CdR	5.0 mg/kg	IV	3 \times TIW

NOTE: The purpose of this study was to compare the tolerability of S-110 to 5-Aza-CdR when administered i.v. on the same schedules. S-110 was administered as molar equivalents of 5-Aza-CdR. Female nude mice were used in this study. Abbreviations: Q7D, every 7 days; BIW, twice weekly; TIW, thrice weekly.

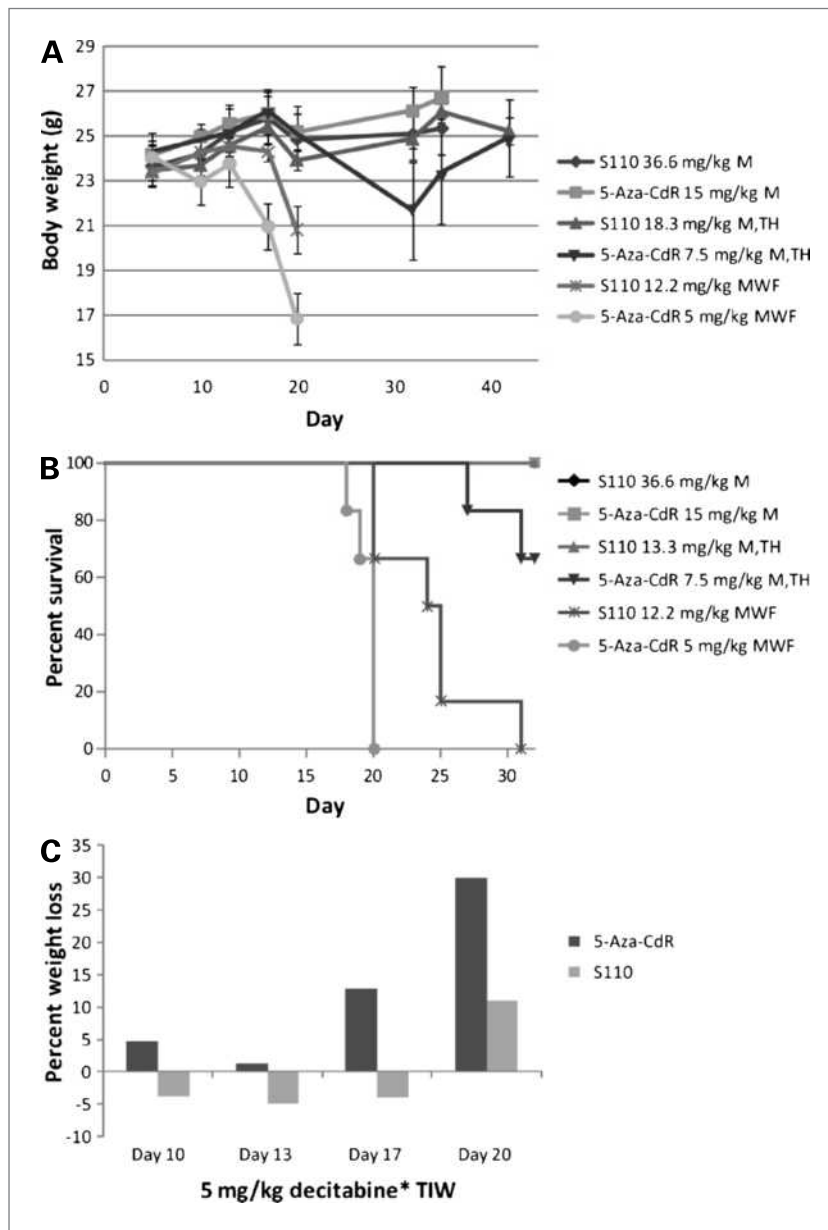


Figure 2. S110 is better tolerated than 5-Aza-CdR as shown by relative body weight (A), percent survival (B), and percent weight loss (C) in normal tumor-free mice. Animals were treated according to the dosages and schedules listed in Table 1. A, six animals per group treated with S110 maintained body weights better and survived longer (B) compared with those treated with 5-Aza-CdR. M, Monday; TH, Thursday; MWF, Monday, Wednesday, and Friday. C, histogram illustrating the effects of S-110 and 5-Aza-CdR treatment on body weight for groups 5 and 6 only, which received 5 mg/kg 5-Aza-CdR or a molar equivalent of S110 thrice weekly. Graphed is the average percent weight loss; therefore, negative values are indicative of weight gain. Animals receiving 5-Aza-CdR began losing weight more rapidly than those receiving S-110. TIW, thrice weekly.

DNA methyltransferases, and reducing cancer cell growth *in vitro*. Moreover, it has the advantage of being less prone to deamination by cytidine deaminase, making it a promising alternative to 5-Aza-CdR.

To compare the *in vivo* tolerability of S110 to that of 5-Aza-CdR, six groups of six tumor-free female nude mice each were randomly assigned to varying schedules according to Table 1. The groups were paired up so that S-110 was administered at molar equivalents of 5-Aza-CdR at the same frequencies in groups 1 and 2, groups 3 and 4, and groups 5 and 6. We first compared the tolerability by examining the relative body weights throughout the study (Fig. 2A). Animals in groups 1

and 2 maintained or steadily gained body weight throughout the course of the study and were euthanized after 36 days. In groups 3 and 4, animals receiving S-110 also maintained or steadily gained weight, whereas the average body weight of those receiving 5-Aza-CdR began to decline after the first week of dosing. Although the average body weight of both groups 5 and 6 rapidly decreased after the first week of dosing, eventually leading to death, those receiving S-110 maintained a healthy body weight slightly longer than animals receiving 5-Aza-CdR. It is of interest that despite the same total weekly dosage of 5-Aza-CdR or S110, both drugs cause more toxicity when given in smaller doses and higher

frequency. In all three paired comparisons, S110 was better tolerated than 5-Aza-CdR in terms of weight change.

We then examined the percent survival (Fig. 2B) of the treated mice. There were no deaths in groups 1 and 2; however, two animals in group 4 died. All animals in group 3 survived, whereas all animals in groups 5 and 6, which received molar equivalents of S110 or 5-Aza-CdR thrice weekly, respectively, died before the completion of the study. However, animals receiving S-110 (group 5) survived 5 to 10 days longer on average than those receiving 5-Aza-CdR (group 6). Lastly, we compared the percent weight loss in groups 5 and 6 (Fig. 2C). Graphed is the average percent weight loss; therefore, negative values are indicative of weight gain. Animals receiving 5-Aza-CdR began losing weight faster than those receiving S-110; the later did not lose weight until approximately day 20 of the study. Taken together, S110 induced less weight loss and mortality than 5-Aza-CdR and is therefore more favorably tolerated in tumor-free mice.

S110 is effective at slowing tumor growth and inhibiting DNA methylation when delivered *i.p.* EJ6 cells were inoculated *s.c.* into the right and left back of female BALB/c athymic nude-Foxn1nu mice. Mice were randomly divided into three groups, and after 2 to 3 weeks, treatments were initiated. Five mice were injected *i.p.* with PBS and 16 mice each were injected with 5-Aza-CdR at 5 mg/kg of mouse weight and S110 at 10 mg/kg of mouse weight. One mouse treated with 5-Aza-CdR died on day 6 before the harvest. Our previous experiment showed that S110 at 10 mg/kg was an effective dose at reducing DNA methylation and retarding tumor growth, and caused roughly the same level of toxicity as 5-Aza-CdR (data not shown).

We studied the potential action of S110 on inhibiting tumor growth. We found that although neither 5-Aza-CdR nor S110 reduced the tumor sizes by the end of the treatment compared with the original sizes on day 0, they both retarded the growth rate of tumors on average compared with the PBS treatment (Fig. 3A).

Real-time RT-PCR analysis of the tumor tissues revealed that S110 was effective *in vivo* at reactivating the expression of the *p16* gene, which is heavily methylated in the parent EJ6 cells (Fig. 3B). In addition, S110 at 10 mg/kg, which was at roughly the same molar concentration as 5-Aza-CdR at 5 mg/kg, induced similar levels of *p16* expression as 5-Aza-CdR at 5 mg/kg. We also noticed that unlike *in vitro* studies, there was greater variation within treatment groups. This could be attributed to the different levels of response among different mice or even between the two different tumors on the same mouse. Overall, S110 was effective *in vivo* at reactivating silenced genes and its activity was comparable with 5-Aza-CdR at roughly the same molar concentrations.

In addition, S110 was effective in reducing the level of DNA methylation *in vivo* at the *p16* promoter region

(Fig. 3C). Although the PBS group showed consistent levels of high methylation, variations among different tumors were much more obvious in the 5-Aza-CdR and the S110 groups. On average, the DNA methylation level was roughly 96% in the PBS group, 82% in the 5-Aza-CdR group, and 86% in the 10 mg/kg S110 group (Fig. 3C). Although the changes in magnitude were small, they were statistically significant as determined by the Student's *t* test (Fig. 3C) when compared with the PBS control group. Because Ms-SNuPE only examined the methylation changes at a few CpG sites, a different method such as genomic bisulfite sequencing might reveal more substantial methylation changes than was determined by Ms-SNuPE.

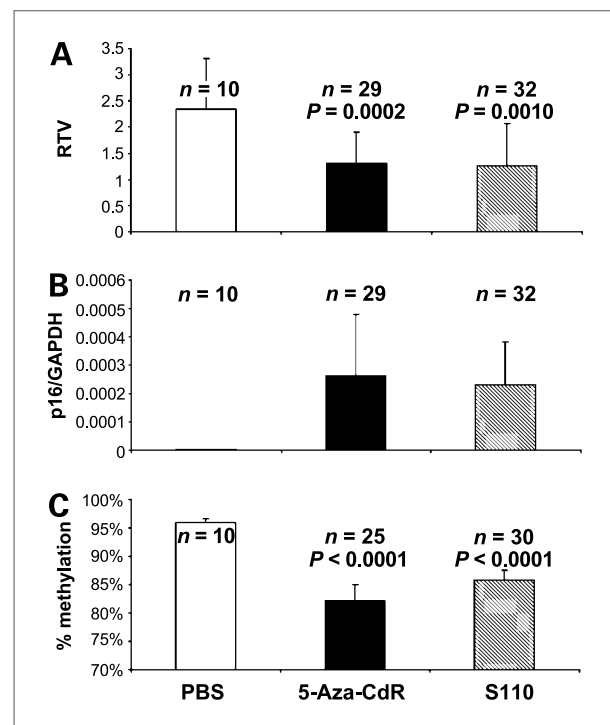


Figure 3. S110 is effective at slowing EJ6 tumor growth *in vivo* (A), inducing *p16* expression (B), and decreasing methylation (C) by *i.p.* administration. Mice were treated by *i.p.* injections of PBS, 5-Aza-CdR at 5 mg/kg, or S110 at 10 mg/kg daily for 6 days. A, tumors were measured with calipers and TVs were calculated by the following formula: $TV = LD^2/2$ (in which *L* is the longest diameter and *D* is the shortest diameter). The fold differences in tumor growth among the various mice groups were calculated using RTVs, which are calculated as follows: $RTV = TV_6/TV_0$, in which TV_6 is the TV in mm^3 at day 6 and TV_0 is the TV in mm^3 at day 0 (initial treatment). B, tumors were harvested on day 6 and total RNA was extracted for RT-PCR analysis. Quantitative real-time RT-PCR analysis for the *p16* gene was done with normalization against the *GAPDH* reference gene. C, tumors were harvested on day 6 and genomic DNA was extracted for Ms-SNuPE analysis. Both 5-Aza-CdR and S110 are effective at reducing DNA methylation at the *p16* gene promoter in the xenografts. The numbers of tumors and *P* values in each group are labeled above the histograms. The changes in percent methylation level in 5-Aza-CdR- and S110-treated groups are statistically significant to the PBS control treatments by the Student's *t* test. Columns, mean; bars, SD.

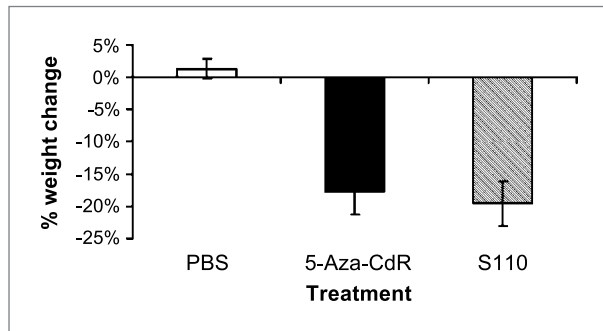


Figure 4. S110 is equitoxic to 5-Aza-CdR *in vivo* by i.p. administration. Mice were weighed at the beginning and the end of treatment to determine toxicity. The percent weight change for each mouse was calculated with the following formula: $[(W_n - W_0)/W_0] \times 100\%$ (in which W_n is the mouse weight on day n). Columns, mean percent weight change; bars, SD. The number of mice in each group is labeled accordingly.

We then estimated the toxicities from the treatments by measuring the weights of the mice at the beginning and the end of the treatment (Fig. 4). Mice treated with PBS did not experience much change in weight, and 5-Aza-CdR and S110 induced similar weight loss in mice. However, the mice treated with S110 appeared healthier compared with the mice treated with 5-Aza-CdR, both by appearance and by their normal level of activity. On the contrary, the mice treated with 5-Aza-CdR appeared cachectic and were not active. These data suggested that S110 could be a promising alternative to 5-Aza-CdR because, at roughly the same molar concentration, the former caused equitoxicity in mice as estimated by mouse weight while remaining comparable in terms of reactivating genes, reducing DNA methylation, and retarding tumor growth in a xenograft model.

S110 is also effective at retarding tumor growth when delivered s.c. To further study the pharmacodynamic action of S110 *in vivo*, we again generated EJ6-xenografted nude mice and divided them randomly into three groups of eight to receive s.c. PBS, 5-Aza-CdR at 5 mg/kg of mouse weight, or S110 at 12.2 mg/kg of mouse weight (equimolar to 5-Aza-CdR). Tumor volumes were measured periodically for the total of 25 days and the average tumor sizes for each group are shown in Fig. 5. Both 5-Aza-CdR and S110 were effective at retarding tumor growth and they were very comparable with each other in their efficacy. Although the tumors did not shrink in size with either the 5-Aza-CdR or S110 treatment, they experienced very minimal growth, whereas the tumors treated with PBS only showed substantial growth. Our results showed that S110 can also be effective when given s.c. In addition, both 5-Aza-CdR and S110 induced much less toxicity as determined by mouse weight changes when given s.c. compared with that with i.p. injections (data not shown); thus, s.c. injection could serve as a different way of delivery that would induce less toxicity.

Discussion

Our results with S110 show that it is an effective DNA methylation inhibitor *in vivo*. It is effective at reducing DNA methylation as determined by Ms-SNuPE and is effective in reactivating *p16* expression as determined by RT-PCR. We also show that it can retard tumor growth in xenografts and that it can be effectively delivered by both i.p. and s.c. routes. Lastly, our data show that S110 is better tolerated than 5-Aza-CdR *in vivo*, suggesting that it can be an attractive alternative for potential clinical use.

Our results show that *p16* is upregulated to different extents in individual mice by the treatments. We notice that although S110 is effective in most tumors, there are individual variations in response to S110, as are also observed with 5-Aza-CdR. It would be of interest to determine which factors contribute to the differences in response. Having this knowledge would also aid us in the future to determine which patients would respond well to DNA methylation inhibitors clinically.

The individual variations we observe also suggest that 5-Aza-CdR and S110 could have secondary effects. Although both drugs are effective DNA methylation inhibitors, they could have actions in addition to inhibiting DNA methyltransferases. Given that at the same level of efficacy, 5-Aza-CdR causes more toxicity in mice by their appearance than S110 in xenograft mice, it is reasonable to hypothesize that either or both drugs can cause changes in addition to affecting DNA methylation. Further studies should be conducted to investigate the actions of these drugs by examining global gene expression changes by microarray analysis. Such studies would illuminate on the global actions of these

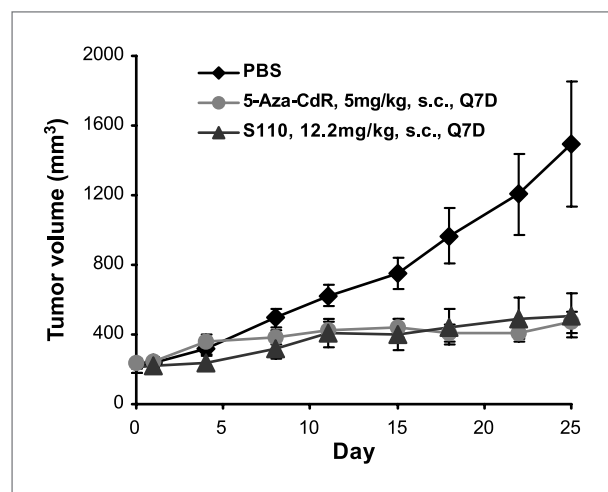


Figure 5. S110 is effective at slowing EJ6 tumor growth *in vivo* by s.c. administration every 7 days (Q7D). Eight mice each were treated by s.c. injections of PBS, 5-Aza-CdR at 5 mg/kg, or S110 at 12.2 mg/kg every 7 days for 25 days. Points, mean of tumor volumes; bars, SD.

drugs and allow us to compare the actions of the different DNA methylation inhibitors side by side.

The more favorable tolerability of S110 compared with 5-Aza-CdR could be due to several possibilities. There could be a differential uptake of the two drugs by different organs. Yoo et al. (27) showed that zebularine, another nucleoside analogue DNA methylation inhibitor, affected different organs in female mice differentially, with the greatest decrease in methylation level in the small intestine and colon. It is therefore reasonable to hypothesize that there are also differential effects of S110 and 5-Aza-CdR on different organs and that could lead to the better tolerability of S110. Until we know the exact mechanisms by which 5-Aza-CdR causes toxicity *in vivo*, we cannot fully answer the question why S110 is better tolerated. The answer is likely contributed by mechanisms at molecular, cellular, and tissue and systemic levels. It would also be of great interest to study the effects of the drugs on other mouse tissues to examine if the drugs have any adverse effects and also to see if different tissues have preferential responses to the drugs.

Our previous study by Yoo et al. (20) showed that S110 is only effective at inhibiting DNA methylation after being cleaved into individual nucleotides and this finding poses the question whether S110 is cleaved first in the circulation before entering individual cells or cleaved later intracellularly after its uptake. From our data that show *i.v.* injections of S110 at equimolar concentrations to 5-Aza-CdR lead to less toxicity in tumor-free mice, one could argue that it is unlikely that S110 was completely cleaved in the circulation before reaching cells given the difference in tolerability. However, this remains a

speculation and more studies are needed in the future to elucidate the exact pharmacodynamic and pharmacokinetic mechanisms of S110.

Given the promising results from our S110 study, further experiments should be conducted to investigate the chemotherapeutic potential and toxicity of S110. In addition to comparing S110 to 5-Aza-CdR at equal molar concentrations, it would be informative to also compare the two drugs at equal toxicity levels because most clinical studies compare the efficacies of different drugs at equal toxicity levels. Having more knowledge of S110 would help us develop more effective DNA methylation inhibitors for chemotherapy in the future.

Disclosure of Potential Conflicts of Interest

P.A. Jones: consultant, Lilly. No other potential conflicts of interest were disclosed.

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