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Report

Antitumor activity of a new orally active organotin compound: a preliminary study in murine tumor models

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The toxicity and antitumor activity of the novel organotin compound triethyltin(IV)lupinylsulfide hydrochloride (IST-FS 29), administered by the oral route, have been evaluated against three transplantable murine tumor models: P388 lymphocytic leukemia, B16F10 melanoma and 3LL Lewis lung carcinoma. Mild and reversible signs of acute toxicity such as behavioral symptoms, weight loss and histological alterations were mainly reported at the highest single dose of 28 mg/kg. Conversely, lower concentrations of compound ranging from 7 to 21 mg/kg did not result in major toxic effects, even after repeated dosing. The antitumor activity studies showed that fractionation dosing, rather than single bolus administration, over 1 week, might prove more active and better tolerated by allowing the achievement of the highest therapeutic total dose of IST-FS 29 (42 mg/kg). Indeed, repeated administrations of IST-FS 29 resulted in marked significant improvement of antitumor activity against B16F10 (50% of tumor volume inhibition, $p = 0.0003$) and, to a greater extent, 3LL (90% of tumor volume inhibition, $p = 0.0001$) tumors. These results indicate that IST-FS 29 might be a suitable candidate as an orally administrable anticancer drug and support its further development in human tumor xenografts. [© 2002 Lippincott Williams & Wilkins.]

Key words: IST-FS 29, murine tumor models, organotin, triethyltin(IV)lupinylsulfide hydrochloride.

Introduction

Tin is a widely distributed metal that can exist in living organisms in different chemical forms. Some organotin compounds, particularly triorganotins such as triphenyltin and tributyltin, have been used worldwide as biocides, fungicides, and stabilizers in polyvinyl chloride and food packaging.[1] In addition, the literature reported that various organotin materials exert *in vitro* antiproliferative activity against tumor cell lines, and retard both the onset and growth of cancer in mice.[2–4]

The organotin derivative, triethyltin(IV)lupinylsulfide hydrochloride (IST-FS 29), was originally selected as one of the most promising agents among a series of organometal complexes.[5] Because of its interesting features such as significant antiproliferative efficacy in a variety of human tumor cell lines *in vitro* and anticancer activity *in vivo*, when administered by parenteral route, IST-FS 29 has been proposed as a novel anticancer agent.[6] This compound is characterized by the presence in its structure of a lupinyl (quinolizidinyl-methyl) moiety. The latter residue, as a consequence of the high lipophilicity combined with basicity (allowing some water solubility after protonation), confers a lipophilic/hydrophilic balance to the compound that proved suitable for ready absorption after oral administration.

The development of orally active compounds could offer evident advantages for clinical use and patient compliance, such as ease of administration and possibility of treatment on an outpatient basis.[7] Since earlier studies have indicated that tin derivatives administered orally are able to decrease tumor growth rate,[8] the acute toxicity and antitumor activity *in vivo*, after oral delivery of IST-FS 29, were investigated in three murine tumor models: P388 lymphocytic leukemia, B16F10 melanoma and 3LL Lewis lung carcinoma.

The present study reports the results of toxicity and tumor growth inhibition following single or

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repeated oral administrations of IST-FS 29 to BDF1 mice transplanted with the above tumor cell lines.

Materials and methods

IST-FS 29

The triethyltin(IV)lupinylsulfide hydrochloride (MW 426.7) was prepared by reacting triethyltinbromide with the quinolizidin-1a-yl methylthiol group, thiolupinine. The initially formed triethyltinlupinylsulfide hydrobromide was converted to the free base, purified and finally converted to hydrochloride.[9] This salt was dissolved in 20% (v/v) ethanol/H_2O at 5.38 mg/ml (12.6 mM; free base MW 390.2, concentration 4.91 mg/ml). The stock solution of IST-FS 29 was freshly diluted with sterile distilled water to reach the planned concentration before each experiment. The structure of IST-FS 29 is reported in Figure 1.

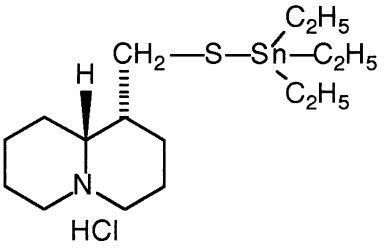

Figure 1. Structure of triethyltin(IV)lupinylsulfide hydrochloride (IST-FS 29).

Animals

C57BL/6, BDF1 (C57BL/6 × DBA/2) and CD1 female mice (6–7 weeks old) were used. Mice (Harlan Nossan, Italy) were allowed a 7-day rest period before experiments. All mice were housed at seven to nine per cage, maintained at 22°C with a 12-h light/dark cycle and fed with a standard diet (4RF-25; Italiana Mangimi, Italy) and water *ad libitum*. Our Institute's Animal Facility adheres to the standards of Federation of European standard Laboratory Animals Associations (FELASA), the care, welfare and health of animals are under the national current regulation, and research protocols are reviewed and approved by the Ethics Committee of the National Institute for Cancer Research, Genoa.

Tumor cell lines

Three murine tumor models have been studied: P388 (lymphocytic leukemia) was obtained from ATCC (Rockville, MD), B16F10 (melanoma) and 3LL (Lewis lung carcinoma) were kindly provided by Dr A Albini (Advanced Biotechnology Center, Genoa) and Dr G Sava (Department of Biochemical Sciences, Trieste), respectively. B16F10 was grown in DMEM medium containing: 1% vitamin, 1% non-essential amino acids, 1% sodium pyruvate and 10% FBS. P388 was maintained *in vivo* by serial i.p. passages in BDF1 mice. 3LL was maintained *in vivo* by serial s.c. flank passages in C57BL/6 mice.

Toxicity studies

Acute toxicity of IST-FS 29 was studied in CD1 mice (21–25 g). Mice were starved for 4–6 h before treatment. Groups of 5+2 satellite animals were given oral (p.o.) administration (400 µl solution/20 g body weight) of single (7, 14 and 28 mg/kg) or repeated (7 mg/kg × 6 times, 14 mg/kg × 3 times, 21 mg/kg given twice) doses of the compound. Control groups received diluted ethanol (EtOH): 2.9% v/v (420 mg/kg) for the doses of 7 and 14 mg/kg or 5.7% v/v (620 mg/kg) for the doses of 21 and 28 mg/kg. Fifteen minutes after dosing the mice were allowed free access to food and water. Over an observation period of 14 days, the behavior and number of survivors were checked twice a day. Body weight (BW) was recorded every 2 days and was used as an index of toxicity. The percentage change in BW was calculated as $\Delta BW\% = [(\text{mean BW on day } x - \text{mean BW on day } 1)/\text{mean BW on day } 1] \times 100$. Satellite animals were sacrificed by CO_2 on day 5 (single dose) or at the end of the experiment (repeated doses). Immediately after autopsy, fresh tissue of the organ explants were fixed and processed for histological analysis.

Histological analysis

Fresh tissues from the organ explants (liver, kidney, spleen, stomach, intestine, heart, lung and brain) of mice treated with single or repeated doses of IST-FS 29 were fixed in B5 solution, processed into paraffin sections (7 µm thick) and stained routinely with hematoxylin & eosin. Histological alterations were defined as: (−) = absent, (+) = mild, (++) = marked

and (+++) = severe, according to intensity and extension of tissue injury.

Antitumor activity studies

The BDF1 female mice (16–19 g) were implanted s.c. with 10^6 cells into the left flank on day 0. After 24 h (day 1) animals were treated orally by single or repeated administrations (400 μl solution/20 g body weight) of IST-FS 29. Keeping constant the final total dose of 42 mg/kg, repeated schedules were: one dose given every day for 6 consecutive days (q1d × 6); three doses given on days 1, 3 and 5 (q2d × 3); two doses given on days 1 and 5 (q5d × 2). Compound was diluted in a water solution containing EtOH, according to doses and schedules of each experiment. Control animals (receiving compound-free EtOH solution) and treated groups included seven to nine tumor-bearing mice. The animals' weights were estimated during the course of the experiment and recorded as ΔBW%. Tumor growth was followed by measurements of tumor diameters every two days with a Vernier caliper and tumor volumes (TV, cm^3) were calculated according to the formula: length × $(width)^2/2$. Mice were sacrificed on day 12 (P388), day 13 (B16F10) or day 15 (3LL), according to tumor type growth and in order to avoid unnecessary suffering due to overdeveloped tumor masses. The activity of IST-FS 29 was assessed as: TVI (tumor volume inhibition)% = 100 – [(mean TV-treated mice/mean TV control mice) × 100].

Statistical analysis

The significance of differences in mean tumor volume of treated versus mean tumor volume of control mice was evaluated by the one-way ANOVA followed by the Neumann–Keuls multiple comparison procedure. p values <0.05 were taken as indicating statistical significance.

Results

Acute toxicity

The toxicity of IST-FS 29 after single or repeated oral administrations was assessed in CD1 mice based on clinical signs, body weight and histology. No toxic death was observed in all the experiments. A dose-dependent change in body weight was recorded; in particular, 10.9% weight loss occurred after single administration of 28 mg/kg on day 5 (nadir), while a small decrease in body weight, ranging from 0.4 to 2.0%, was observed at lower doses (Table 1). Mice weight loss was reversible and increase in body weight was recorded by the end of treatment. Immediately after IST-FS 29 administration at 21–28 mg/kg, mice presented peripheral vasodilatation and some behavioral symptoms, such as increased motor activity. These were followed by restoration to the normal conditions within 20–30 min.Toxicity was also assessed at the histological level on main organ tissues including heart, lung, liver, spleen, kidney, stomach, intestine and brain. Data from the analyses revealed slight inflammatory alterations of the intestinal mucosa and vacuolation of the brain white matter only at the highest dose (Table 1). Animals that received repeated doses of IST-FS 29 exhibited acute behavioral signs of toxicity comparable to those given single doses with the exception of mice from the q1d × 6 treatment schedule. Indeed, the frequency of administration procedure of this last protocol was likely more stressful and in turns more toxic. The histological sections of the organs were processed and checked at the end of the experiments, i.e. about 1 week after the last administration, and were unremarkable.

Antitumor activity of IST-FS 29 after oral administration

Oral antitumor activity of IST-FS 29 was evaluated against three murine tumor models: P388, B16F10

Table 1. Toxic effects induced by IST-FS 29 given p.o. as single dose in CD1 mice (day 5)

Dose (mg/kg)	Liver	Intestine	Brain	ΔBW%[a]
Control (EtOH)	(−)[b]	(−)	(−)	+2.6
7	(−)	small lymphoid clumps	(−)	−2.0
14	(−)	lymphoid clumps	(−)	−0.4
28	venous stasis (+)	big lymphoid formation	vacuolation of the white matter (+)	−10.9

Stomach, lung, heart, kidney and spleen did not show histological alterations.
[a]ΔBW% = [(mean BW on day x − mean BW on day 1)/mean BW on day 1] × 100.
[b](−) = Absent, (+) = mild histological alterations.

Table 2. Antitumor activity of IST-FS 29 given p.o. as single dose against the P388 in BDF1 mice (day 12)

Dose (mg/kg)	TV[a] ± SD	TVI%[b]	ΔBW% (day 5)[c]	*p* value (versus control)[d]
Control (EtOH)	2.66 ± 0.67	–	+0.5	–
7	2.27 ± 0.43	13.5	+3.0	NS
14	1.91 ± 0.40	27.1	+2.9	< 0.05
21	1.81 ± 0.40	31.1	+1.7	< 0.05
28	1.31 ± 0.42	50.0	−5.4	< 0.01

p value by ANOVA = 0.0003.
[a]Tumor volume.
[b]TVI% = 100 − [(mean TV treated/mean TV control) × 100].
[c]ΔBW% = [(mean BW on day *x* − mean BW on day 1)/mean BW on day 1] × 100.
[d]Neumann–Keuls.

and 3LL. The compound was administered to BDF1 mice according to the optimal dosing and schedule selected from previous toxicity studies. Control animals were given the corresponding compound-free ethanol solution.

IST-FS 29 administered to P388-bearing mice at the single dose of 7, 14, 21 and 28 mg/kg induced a significant dose–response effect ($p = 0.0003$). The most relevant activity was achieved at the highest dose of 28 mg/kg providing 50% tumor inhibition and 5.4% body weight reduction (day 5), which completely recovered by the end of the experiment. An intermediate, but still significant, efficacy was found at 14 and 21 mg/kg with 27.1 and 31.1% of TVI, respectively. Minimal activity was found at the lowest concentration of 7 mg/kg. These results are summarized in Table 2.

Since in the above tumor model the concentration of 28 mg/kg was more effective, but also more toxic, this dose was no longer used in the following investigations.

The antitumor activity of IST-FS 29 was then studied against the B16F10 melanoma, at first by single administration of 7, 14 and 21 mg/kg. B16F10 showed slight responsiveness to single doses (TVI: 3.2–17.5%) as reported in Table 3. In order to improve the therapeutic index of IST-FS 29, the total dose of 42 mg/kg was fractionated into different administrations according to treatment schedules previously described. A statistically significant dose–response relationship, with no evident toxicity, was reached by these treatment protocols ($p = 0.0003$). The advantage of the repeated administrations emerged by looking at the q2d × 3 and q5d × 2 schedules which revealed equivalent inhibition of tumor growth (about 50%). Conversely, the q1d × 6 schedule was almost ineffective (TVI: 14.1%) and less well tolerated (Table 4).

The study next moved to investigate whether the most active treatment protocols in B16F10 might translate into a significant antitumor activity against the 3LL murine tumor model as well. As shown in Table 5, IST-FS 29 exhibited higher efficacy towards this tumor type and confirmed the equivalent level of activity of both schedules as demonstrated by TVIs of 88.5 and 94.8% ($p = 0.0001$). It is worth mentioning that the tumor volumes of four out of eight animals from the q2d × 3 treatment scheme and of six out of eight mice from the q5d × 2 protocol were barely detectable, and mice did not develop measurable tumors following 7 days from the end of the experiment (data not shown).

Table 3. Antitumor activity of IST- FS 29 given p.o. as single dose against the B16F10 in BDF1 mice (day 13)[a]

Dose (mg/kg)	TV ± SD	TVI%	ΔBW% (day 5)
Control (EtOH)	1.53 ± 0.36	–	+3.1
7	1.48 ± 0.48	3.2	+3.0
14	1.36 ± 0.43	11.0	+4.5
21	1.26 ± 0.42	17.5	+1.7

p value by ANOVA = NS.
[a]See footnotes to Table 2.

Discussion

A number of observations have underlined the pharmacological antiproliferative relevance of some organometal complexes as potential anticancer drugs.[2,3] Among a variety of gold and tin compounds, we recently identified and studied a tin derivative, the triethyltin(IV)lupinylsulfide hydrochloride, to be selected for therapeutic purpose.[5]

Our recent findings *in vitro* and, preliminarily, *in vivo* have confirmed the interest of this molecule as a potential antitumor agent. IST-FS 29 exhibited antiproliferative activity against a panel of human cell lines and antitumor potency when administered

Table 4. Antitumor activity of IST-FS 29 given p.o. as repeated doses against the B16F10 in BDF1 mice (day 13)[a]

Single dose (mg/kg)	Treatment schedule	Total dose (mg/kg)	TV ± SD	TVI%	Max ΔBW%[b]	*p* value
Control (7)			1.77 ± 0.45	–	−1.5	
	(q1d × 6)					NS
7		42	1.52 ± 0.42	14.1	−0.5	
Control (14)			1.64 ± 0.42	–	−0.6	
	(q2d × 3)					< 0.01
14		42	0.81 ± 0.48	50.6	−7.1	
Control (21)			1.70 ± 0.45	–	+0.2	
	(q5d × 2)					< 0.01
21		42	0.84 ± 0.28	50.6	−2.7	

p value by ANOVA = 0.0003
[a]See footnotes to Table 2.
[b]Max ΔBW% = maximal weight loss after the last dose.

Table 5. Antitumor activity of IST-FS–29 given p.o. as repeated doses against the 3LL in BDF1 mice (day 15)[a]

Single dose (mg/kg)	Treatment schedule	Total dose (mg/kg)	TV ± SD	TVI%	Max ΔBW%[b]	*p* value
Control (14)			2.44 ± 0.76		+2.9	
	(q2d × 3)					< 0.01
14		42	0.28 ± 0.36	88.5	−6.7	
Control (21)			2.73 ± 0.99		+5.3	
	(q5d × 2)					< 0.01
21		42	0.14 ± 0.27	94.8	−6.5	

p value by ANOVA = 0.0001.
[a]See footnotes to Table 2.
[b]Max ΔBW% = maximal weight loss after the last dose.

i.p. in tumor-bearing mice.[5,6] The high toxicity and poor solubility in aqueous solution of various organotin derivatives described in the literature, often prevented their use *in vivo* except for a small minority of them.[10,11] In order to mitigate undesirable side effects *in vivo*, IST-FS 29 was designed to obtain a molecular structure able to assure good chemical stability and reduced reactivity of the tin atom.

The great majority of cytotoxic agents easily degrade in the gastrointestinal environment or possess poor water solubility that limits their use to parenteral formulations. The possibility of administering IST-FS 29 by the oral route led us to explore the toxicity, first, and antitumor efficacy, thereafter, in murine tumor models. The most noteworthy toxic effects only resulted at the highest dose and, as previously reported for other triorganotins, neurotoxicity appeared the limiting one.[12,13] Indeed, data from the literature suggest that triorganotin may affect the central and peripheral nervous system likely through the inhibition of Ca^{2+}-ATPase activity in brain, by interacting with calmodulin,[14] and the elevation of the cytosolic and synaptosomal free Ca^{2+} concentration.[15] In addition, evidence has been reported that *in vivo* exposure to triethyltin induced myelin vacuolization within the white matter,[16] resulting in CNS edema.[12]

In the current study, brain white matter vacuolation was observed at the highest concentration of IST-FS 29 (28 mg/kg). On the other hand, no detectable toxicity was reported at lower doses, except for a slight inflammatory reaction in the gastrointestinal mucosa. This toxicity was only observed on day 5 after single dose administration and was reversible. Moreover, by taking into account the repeated dose schedules, good recovery and lack of cumulative toxicity of the animals was recorded. Indeed, histological sections from animals sacrificed at the end of the experiments (about 1 week from last administration) did not reveal presence of tissue alteration even in the target organs such as kidney, liver, intestine and brain.

In our tumor models a clear potentiation of the antiproliferative effect and good tolerability of IST-FS 29 was obtained by repeated oral administrations over 1 week which allowed the achievement of the highest therapeutic total dose (42 mg/kg). Since the drug schedule represents an important factor in determining compound activity and toxicity, different schedules have been studied to optimize IST-FS 29 treatment and achieve greater efficacy. The

multiple dosing experiments indicated that dosage fractionation permitted a higher total drug concentration, which gave an improvement of the antitumor activity. The potentiation of the IST-FS 29 cytotoxic effect was obtained by the q2d × 3 and q5d × 2 schemes as demonstrated in B16F10 and 3LL tumors. However, shorter intervals between doses, such as the q1d × 6 schedule, seemed to decrease the efficacy, and were almost ineffective and less tolerated in the B16F10 tumor-bearing mice. These observations suggest that a more stressful treatment intensity (every day for 6 days) or a more rapid metabolism of the compound (due to lower single dosage) might be the consequence of this reduced antitumor efficacy. Investigations of the interaction of IST-FS 29 with the cellular enzymatic components may lead to additional insights into the cellular pharmacology of this novel agent. Indeed, in spite of IST-FS 29's potential for inhibiting cancer growth, the mechanism of action has not yet been completely elucidated and warrants further investigation. Data from the literature and our recent results showed the possibility that compounds such as the present one may act through energetic metabolism interactions rather than DNA binding or apoptotic induction.[17–19] Further extension of the antitumor *in vivo* study will be performed to clarify the potentiality of this compound against human tumor xenografts as well.

Conclusions

The results of this preclinical investigation suggest that IST-FS 29 could be a favorable candidate as an orally administrable anticancer drug. In addition, due to its lipophilic properties, as reflected by rapidly crossing the physiological barriers, and significant cytotoxic activity, IST-FS 29 may prove suitable for a wide therapeutic range and may also be proposed as a novel agent for neoplasms localized to the brain.

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