Invest New Drugs
DOI 10.1007/s10637-008-9176-6

PRECLINICAL STUDIES

p53-dependent antiproliferative and antitumor effect of novel alkyl series of diorganotin(IV) compounds

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Received: 12 August 2008 / Accepted: 3 September 2008

Summary *Purpose:* A series of diorganotin(IV) dichloride complexes of *N*-(2-pyridylmethylene)arylamine (nitrogen-chelating ligands) have been synthesized and characterized. The present study was carried out to investigate the comparative anti-proliferative and anti-tumor effect of $Me_2SnCl_2.L^1$ (OTC-1), $Et_2SnCl_2.L^2$ (OTC-2) and $^nBu_2SnCl.L^2$ (OTC-3) in combination with X-rays (1.5 Gy). *Method:* The cytotoxicity of these diorganotin(IV) compounds was studied in human peripheral lymphocytes and the antitumor activity was assessed in Dalton's lymphoma cells. The involvement of proteins that regulate cell cycle and apoptosis was investigated to elucidate the mechanism of their action. *Results:* 5 mg kg^{-1} of OTC-3 showed better antiproliferative and antitumor activity than OTC-1 and OTC-2, both as alone or in combination with X-rays. The maximum enhancement of exchange aberrations and the level of p53 and p16 proteins were observed in the OTC-3 treated samples. Upregulated expression of p53 caused a significant down-regulated transcriptionally repression of Survivin in OTC-3 treated human lymphocytes. *Conclusion:* It could be possible that after treatment with either OTC-3 alone or in combination with X-rays the Dalton's lymphoma cells may die apoptotically after inducing initial delay in cell cycle and thereby survivality of mouse bearing Dalton's Lymphoma cells was increased significantly.

Keywords Diorganotin(IV) · Antiproliferation · Anti-tumor · Apoptosis

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Introduction

The biological activity of organotin(IV) compounds is well known owing to their practical applications as fungicides, bactericides, biocides and pesticides [1, 2]. However, one of the fields that have been more studied and reviewed is the activity of such compounds against cancer [1, 3]. The organotin(IV) compounds that were first tested were those that were available or easily synthesized, like tri- or diorganotin(IV) halides. It was reported that diorganotin (IV) oxides and hydroxides, which either contain tin-oxygen bond or are capable of forming such a bond upon hydrolysis, have antitumor potential [4, 5].

Diorganotin(IV) compounds, R_2SnCl_2 are often tetrahedral, and when appropriate nitrogen-chelating ligands are co-ordinated to the central metal, octahedral complexes $R_2SnCl_2{\cdot}L$ (L=bidentate ligand) are obtained [6]. Diorganotin(IV) compounds structurally resemble the active platinum compounds, i.e. cisplatin and carboplatin, and consequently a large number of such complexes have been tested for antitumor activity. Three primary factors are involved in the structure–activity relationship for organotin (IV) derivatives $R_nSnX_{4-n}(L)_x$: the nature of the organic group R, of halide or pseudohalide X, and of donor ligand L. Examination of the structures of organotin(IV) com-

pounds containing a N-donor atom when tested for antitumor activity revealed that in the active Sn complexes the average Sn–N bond lengths were >2.39A°, whereas the inactive complexes had Sn–N bonds <2.39A°. This implies that predissociation of the ligand may be an important step in the mode of action of these complexes, while the coordinated ligand may favour transport of the active species to the site of action in the cells, where they are released by hydrolysis [1]. A structural correlation with biological activity for diorganotin(IV) complexes has shown that active species are associated with complexes having Sn–N bonds longer than 2.39A° which in turn determines the formation of a tin–DNA complex [7]. In view of these, a series of diorganotin(IV) dichloride complexes of *N*-(2-pyridyl-methylene)arylamine (nitrogen chelating ligands) have been synthesized and characterized on the basis of IR, NMR and ^{119}Sn-Mössbauer studies [8, 9]. The observed Sn–N bond lengths in $Me_2SnCl_2{\cdot}L^1$, $Et_2SnCl_2{\cdot}L^2$ and $^nBu_2SnCl_2{\cdot}L^2$ is Sn–N(1)=2.452 (6) and Sn–N (2)= 2.559 (6) which is >2.39A° and therefore, better formation of tin–DNA complex can be expected. In line with these developments, the anti-proliferative and cytotoxic effect of $Me_2SnCl_2{\cdot}L^1$, $Et_2SnCl_2{\cdot}L^2$ and $^nBu_2SnCl_2{\cdot}L^2$ have been investigated both in vivo and in vitro [8–10]. The data suggest that the $^nBu_2SnCl_2{\cdot}L^2$ has better antiproliferative and antitumor activity than the other two but it shows higher toxicity to mice [9]. It was shown earlier that the antitumor activity of $Et_2SnCl_2{\cdot}L^2$ was improved after depleting endogenous glutathione (GSH) by buthionine sulfoximine (BSO) [10], however, such treatment did not show any effect with $^nBu_2SnCl_2{\cdot}L^2$ [9].

Therefore, the present study was carried out to investigate the comparative anti-tumor and anti-proliferative effect at dose much below than the LD_{10} concentration for $Me_2SnCl_2.L^1$, $Et_2SnCl_2.L^2$ and $^nBu_2SnCl.L^2$ which were 28, 20 and 14 mg kg^{-1}, respectively. However, an attempt was made to improve the antitumor potentiality of these diorganotin(IV) compounds by combining it with X-rays instead of increasing the dose of diorganotin(IV) compounds. It has been observed previously that the radiation and chemical combined therapy has led to improved local control and disease-free survival [11]. Furthermore, the involvement of proteins that regulate cell cycle and apoptosis was investigated to elucidate the mechanism of their action.

Materials and methods

Diorganotin(IV) compounds, $R_2SnCl_2.L$

The diorganotin(IV) dichloride complexes of *N*-(2-pyridyl-methylene)arylamine, e.g., $Me_2SnCl_2.L^1$ (OTC-1), $Et_2SnCl_2{\cdot}L^2$ (OTC-2) and $^nBu_2SnCl_2.L^2$ (OTC-3) were synthesized as previously described [8]. The stock solutions of OTC-1, OTC-2 and OTC-3 were prepared in 5% (*v*/*v*) ethanol/water keeping a concentration of 3 mg ml^{-1}. The stock solutions of OTCs were freshly diluted with distilled water to reach a planned concentration before each experiment.

Chemicals

DL-Buthionine-S,R-sulphoximine (BSO), 5-bromodeoxyuridine, Nonidet P-40, sodium dodecylsulphate, aprotinin, and reduced glutathione (GSH) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Hoechst 33258 was obtained from Roche Chemicals (Germany). Giemsa stain was obtained from BDH Chemicals Ltd. (Poole, UK). The culture medium Rosewell Park Memorial Institute (RPMI) 1640, fetal calf serum, antibiotics penicillin and streptomycin and mitogen phytohaemagglutinine were obtained from Gibco, USA.

Primary Antibodies p53-Abs (DO7+Bp53-12), p21 Ab-11, Survivin Ab-1 and β-actin (anti-actin ACTN05) were obtained from Neomarker (Fremont, CA, USA). Secondary antibody rabbit antimouse IgG-Alkaline phosphatase conjugate and substrate for alkaline phosphatase, Bromo-4-chloro-3-indolyl Phosphate/Nitro Blue Tetrazolium (BCIP/NBT) were obtained from Bangalore Genie (Bangalore India). Other chemicals used in this study were of analytical grade from reputed manufacturers.

Collection of human blood and treatment

Heparinized peripheral blood from five healthy male donors was used immediately after venipuncture. The lymphocytes are in G_0 stage. Rules of 'Ethical Guidelines for Biomedical Research on Human Subjects' of the Indian Council of Medical Research, India were followed in all the experiments. One of the three diorganotin(IV) complex (2 μg ml^{-1}) was added to the 1 ml blood for 2 h. At the end of 2 h treatment, the treated samples were washed twice with pre-warmed medium and were irradiated with Faxitron Cabinet X-ray Systems (Model No. 43855D, 110 kVp, 3 mA, Beryllium window thickness 0.76 mm) at the dose rate of 1.5 Gy min^{-1}. All the samples were kept at 37°C for an hour after irradiation to allow normal cellular repair before setting up cultures in RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum following phytohaemagglutinin stimulation and incubated at 37°C. To obtain differential sister chromatid staining, 5 μg ml^{-1} 5-bromodeoxyuridine was added to the cultures at the time of initiation. Cells were harvested at 72 h and colcemid (0.1 μg ml^{-1}) was added 3 h prior to that.

Cells were treated with prewarmed (37°C) hypotonic solution (0.075 M KCl) for 15 min and fixed in acetic acid and methanol (1:3). Slides were prepared by air drying method.

Differential staining

Differential staining was carried out following the method of Goto et al. [12]. Slides were treated 10 min with Hoechst 33258 (50 μg ml^{-1}) at room temperature in dark, rinsed in distilled water, mounted in 2× SSC (NaCl–Na–Citrate, pH 6.8) and kept in sunlight for 30–40 min depending on the intensity of sunlight. After rinsing in distilled water, slides were stained in 2% Giemsa (BDH Chemicals, UK) for 3–4 min, air-dried and mounted on synthetic medium.

Antitumor activity

The antitumor activity of diorganitin(IV) compounds was assessed in Dalton's lymphoma (DL) cells, in a different set of experiments using 2–3 months old male Swiss-albino mice. They were maintained in the laboratory in community cages in controlled-temperature room (20°±2°C), with controlled lighting (12 h light/12 h dark). Standard mouse diet (NMC Oil Mills Ltd., Pune, India) and water *ad libitum* were used in all experiments. The rules of the Institutional Animal Care and Use Committee were strictly followed during the whole experiment and steps were taken to protect the welfare of the experimental animals.

An inoculum size of 10^6 cells per mouse was used, and the animals were maintained by serial intraperitoneal transplantation. The day DL cells were transplanted was considered day '0'; diorganotin(IV) compound (5, 10 and 20 mg kg^{-1}) was administered on the first, fifth and ninth day. In case of radiation, 10^6 Dalton's lymphoma cells were irradiated with 1.5 Gy with Faxitron Cabinet X-ray Systems at the dose rate of 1.5 Gy min^{-1} and then transplanted intraperitoneally. While diorganitin(IV) compounds was given in the combined treatment, the lower concentration (5 mg kg^{-1}) was administered on the first, fifth and ninth day after transplantation of 1.5 Gy irradiated Dalton's lymphoma cells.

Western blot analysis

The expressions of p53, p21 and Survivin proteins after diorganotin(IV) treatment was analyzed by immunoblotting. Lymphocytes were isolated by density gradient centrifugation in Histopaque (Sigma) as described by McFee et al. [13]. The monocyte cell layer was washed in phosphate-buffered saline and about 2×10^6 viable lymphocytes were added to 1 ml RPMI culture medium in a sterilized small flat bottom 25 ml glass beaker. Diorganotin (IV)-treatment was given for 2 h and during treatment the samples were kept at 37°C. Cells were lysed 6 h after the treatment in radioimmuno-precipitation buffer (0.1% SDS, 2 mM EDTA, 1% NP-40, 1% sodium deoxycholate, 50 mM sodium fluoride and 100 U/ml aprotinin). After 30 min of incubation on ice, the cell lysates were centrifuged for 15 min at 4°C and the amount of protein was determined using the bicinchonic acid protein assay [14]. Equal amount of protein (60 μg) from each sample was loaded in each well, and equal loading was verified by immunoblotting with actin antibodies (anti-actin ACTN05; Neo-Markers). Electrophoresis was performed in 12% polyacrylamide separating gel and 5% stacking gel. Proteins were transferred to a 0.45 μm nitrocellulose membrane (Sigma) following the standard protocol. The membranes were probed with a 1:1,000 dilution of a mouse monoclonal antibody against p53 Ab-8 and anti-p21 Ab-6 (Neo-Markers). Blots were washed three times for 10 min each in TBST buffer pH 7.6 (1 M Tris Cl, 5 M NaCl and 0.05% Tween 20) and incubated with secondary antibody (alkaline-phosphatase conjugated anti-mouse IgG, 1:2,000; Bangalore Genei, Bangalore, India) for 1 h at room temperature. After extensive washing, the blot was immersed in 4 ml substrate solution of BCIP/NBT (Bangalore Genei, Bangalore, India). Sufficient staining was obtained within 15 min. The whole experiment was repeated twice.

DNA fragmentation assay

The day Dalton's lymphoma cells were transplanted was considered day '0'; diorganotin(IV) compound (5 mg kg^{-1}) was administered on the first, fifth and ninth day. In case of radiation, 10^6 Dalton's lymphoma cells were irradiated with 1.5 Gy with Faxitron Cabinet X-ray Systems at the dose rate of 1.5 Gy min^{-1} and then transplanted intraperitoneally. While diorganitin(IV) compound was given in the combined treatment, it was administered on the first, fifth and ninth day after transplantation of 1.5 Gy irradiated Dalton's lymphoma cells.

On 10th day the cells were harvested at different time-points, washed once with ice-cold PBS, and resuspended in 250 μl lysis buffer [10 mM Tris–HCl, pH7.6, 20 mM EDTA, pH 8.0 and 0.5% (*w*/*v*) Triton X-100]. After centrifugation at 12,000 rpm for 5 min, the supernatant was extracted once with phenol/chloroform (1:1) and once with chloroform/isoamyl alcohol (24:1). DNA was precipitated with sodium acetate (pH5.2) at −20°C overnight. The DNA was then pelleted and subsequently digested with DNase-free RNaseA (Amersham Biosciences UK Ltd., Little Chalfont, Buckinghamshire, UK) at 37°C for 20 min. The extracted genomic DNA fragments were fractionated by 2% agarose gel and were further visualized by ethidium bromide under UV-light.

Scoring and statistical analysis

Slides were randomly coded. For scoring cell cycle kinetics, metaphases from human lymphocytes were categorized as in first, second and subsequent division cycles based on their differential staining patterns. Chromosome aberrations (CAs) were scored from first cycle metaphases (M1) only and they were: exchanges including dicentrics and rings (with or without fragments); deletions and chromatid breaks. Statistical significance of the difference between control and treated groups for the frequency of M1 cells and aberrant metaphases, was evaluated using 2×2 contingency χ^2-test.

Results

For scoring cell cycle kinetics in human PBLs, metaphases were categorized as in different cycles based on their differential staining pattern. It was possible to distinguish unequivocally the number of divisions in the presence of BrdU. Cell cycle progression and CA were scored simultaneously and the data are presented in tabular form. Negative control data are also shown. For antitumor activity, low doses of (5 mg kg^{-1}) diorganotin(IV) compounds were used.

Effect of diorganotin(IV) complexes and X-rays on cell proliferation

Diorganotin (IV) complexes and 1.5 Gy X-ray induced first cycle metaphases (M1) in human PBLs were studied as positive controls to diorganotin (IV) complexes+X-ray treated samples and the data are presented in Table 1. Delay in cell cycle progression was measured in terms of increase in the frequency of first-cycle metaphases (M1) following treatment in comparison to that of untreated controls. Basic cell cycle progression varied among the donors and therefore, the mean data can be considered to measure the delay in cell cycle. X-rays alone and each diorganotin(IV) complexes alone induced significant delay in cell cycle. The extent of delay induced by OTC-3 was the most among the three tin-complexes diorganotin(IV) at 72 h harvested samples. The amount of delay induced by both OTC-1 and OTC-2 was almost similar. This induction

Table 1 Data showing the effect of diorganotin(IV) (2 μg ml^{-1}) alone or in combination with X-rays on the cell kinetics, aberrant metaphases in human lymphocyte cultures

Experimental condition	TM	M1%	Abt. M	Percentage of		
			(TM)	Deletion	Chd bk	Exchanges
Untreated	207	47	2 (106)	0	2	0
	241	45	3 (201)	0	3	0
Mean±SEM		46±1	2.5±0.5			
1.5 Gy	164	63	30 (109)	10	2	18
	133	58	34 (127)	11	4	17
Mean±SEM		61±2.5*	32±2	10.5±0.5	3±0.5	17.5±0.5
OTC-1	153	58	6 (121)	0	6	0
	135	55	4 (091)	0	5	0
Mean±SEM		56.5±1.5*	5±1		5.5±0.5	
OTC-1+1.5 Gy	123	69	39 (109)	14	15	22
	114	65	42 (104)	16	17	26
Mean±SEM		67±2*	40.5±1.5	15±1	16±1	24±2
OTC-2	183	52	4 (109)	0	7	0
	235	54	5 (132)	0	5	0
Mean±SEM		53±1	4.5±0.5		5.5±0.5	
OTC-2+1.5 Gy	107	67	38 (91)	16	15	24
	110	72	41 (143)	18	17	28
Mean±SEM		69.5±2.5*	39.5±1.5	17±1	16±1	26±2
OTC-3	138	66	5 (112)	0	5	0
	166	60	7 (124)	2	5	0
Mean±SEM		63±3**	6±1.0	1±0	5±0	
OTC-3+1.5 Gy	112	90	43 (103)	14	20	30
	124	96	41 (125)	11	16	33
Mean±SEM		93±1.5**	42±1*	12.5±1.5	18±2	31.5±1.5

*Significant at $p<0.05$ and ** at $p<0.01$ 2×2 contingency χ^2-test compared with respective control
TM Total metaphases, *M1* 1st cycle metaphases, *Abt. M* Aberrant metaphases, *Chd bk* chromatid break

of delay in cell cycle was increased significantly while when combined treatment of diorganotin(IV) complexes and X-rays was given together. The highest induction of delay was shown while when radiation combined with OTC-3.

Effect of diorganotin(IV) complexes and X-rays on CAs

The induction of CA by the present diorganotin(IV) complexes was low and showed almost similar in frequency, however the induction was significantly more while 1.5 Gy X-rays was combined to diorganotin (IV) complexes (Table 1). These diorganotin(IV) complexes mostly induced chromatid breaks without any exchanges and in case of X-rays alone deletions and exchanges were induced predominantly. In the combined treatment the frequency of aberrant metaphases and all types of aberrations was increased. The maximum enhancement of exchange aberrations was shown with OTC-3.

Antitumor activity

The treated/control values are shown in Table 2. Data indicate the T/C value was improved in a dose dependent fashion for both OTC-1 and OTC-2 but for OTC-3 the lower concentration was better since at higher concentration 11 mice were died prematurely. However, the T/C value was improved significantly for all the diorganotin(IV) complexes while combined with 1.5 Gy X-rays. The X-rays alone also showed high activity on DL cells. The T/C value was 140% when 1.5 Gy was given only once to DL cells. The best value of T/C was shown while radiation combined with OTC-3.

Apoptosis induction

To validate the apoptotic induction from treatment of diorganotin(IV) complexes, DNA fragmentation analysis was performed in Dalton's lymphoma cells. A typical laddering pattern, which is believed to occur at the later stage in apoptosis, is shown in Fig. 1a and b. The laddering pattern is only seen after 24 h of treatment with OTC-3 alone or in combination with 1.5 Gy X-rays. This experiment was repeated twice.

Western blot analysis

Representative results of the Western blot analysis are illustrated in Fig 1c. β-Actin was included as an internal indicator in all analyses to control for potential discrepancy in sample loading. The level of p53 and p21 proteins were more in the OTC-3 treated sample than OTC-1 and OTC-2 treated sample. In case of surviving protein the level was more in OTC-1 and OTC-2 treated sample than OTC-3. The level of all the proteins was analyzed after 6 h of treatment.

Discussion

This study was carried out to evaluate the comparative cytotoxic and antitumor effect of the diorganotin(IV) complexes (methyl-, ethyl- and *n*-butyl) at lower concentration with or without X-rays in combination. In the previous study, it was shown that OTC-3 has better antiproliferative and antitumor activity than OTC-1 and OTC-2, however, it showed more toxicity since the dose used was very close to its LD_{10} value [9].

Table 2 Antitumor activity of alkyl series of diorganotin(IV) compounds with or without X-rays against Dalton's lymphoma transplanted in mice

Treatment	Dose mg kg^{-1}	No. of Animals	Tin treatment	Premature death[a]	Range survival	Median survival days	T/C % days
Untreated	–	20	–	–	13–18	15	–
OTC-1	5	10	1st, 5th, 9th		18–24	20	133
OTC-2		10	1st, 5th, 9th		12–23	19	127
OTC-3		10	1st, 5th, 9th		23–30	27	180
OTC-1	10	12	1st, 5th, 9th	–	18–25	22	147
OTC-2		12	1st, 5th, 9th		16–23	17	113
OTC-3		14	1st, 5th, 9th		22–35	28	186
OTC-1	20	12	1st, 5th, 9th		19–31	23	153
OTC-2		12	1st, 5th, 9th		20–22	21	140
OTC-3		20	1st, 5th, 9th	11	11–26	11	73
X-rays	1.5Gy	10	–		17–26	21	140
Radt+OTC-1	1.5Gy+5	10	1st, 5th, 9th		20–30	26	173
Radt+OTC-2		10	1st, 5th, 9th		20–31	31	206
Radt+OTC-3		10	1st, 5th, 9th		29–38	35	233

[a] Mice died before completing the tin treatment

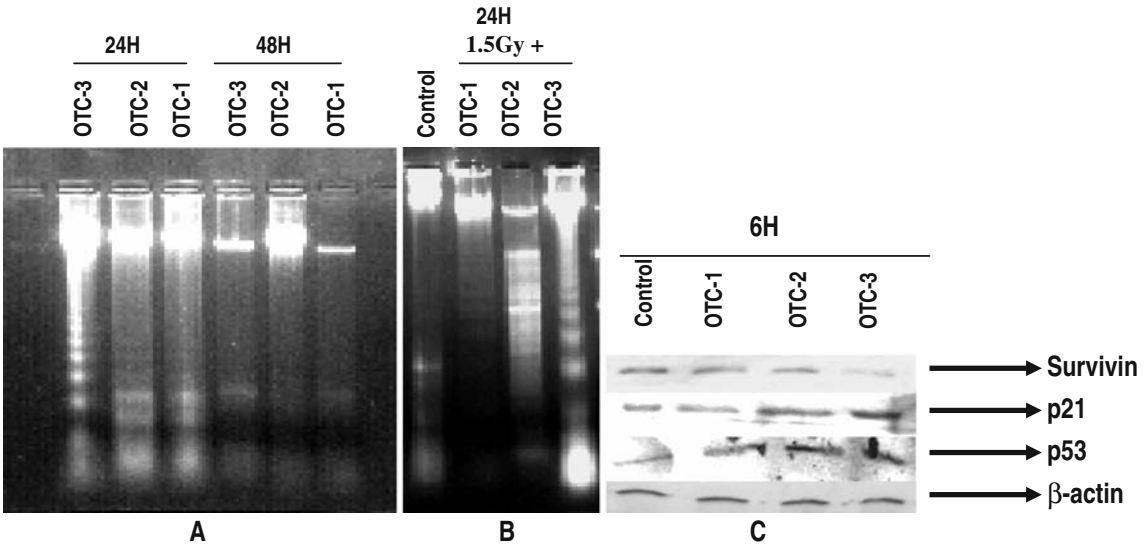

Fig. 1 Induction of apoptosis in Dalton's lymphoma cells and immunoblotting analysis of different proteins in human lymphocytes treated with 2 μg ml^{-1} diorganotin(IV) complexes. DNA-laddering assay shows the typical pattern of oligonucleosomal-sized fragments of about 200 base pair length in diorganotin(IV) complexes alone (**a**), and X-rays+diorganotin(IV) complexes (**b**) treated cells. **c** Immunoblotting analysis of p53, p21 and surviving proteins lysed 6 h after the treatment with diorganotin(IV) complexes for 2 h

The present study demonstrated that 5 mg kg^{-1} diorganotin (IV) complexes could block the cell cycle progression significantly and also demonstrated its potential as an anticancer activity. Like earlier studies [9] OTC-3 showed better antiproliferative and antitumor activity than other two diorganotin(IV) compounds (OTC-1 and OTC-2). In order to improve the efficacy of the diorganotin(IV) compounds at lower doses in treating cancers the treatment of diorganotin(IV) compounds is combined with X-rays. It was shown that in the combined treatment, the frequency of M1 cells and all types of aberrations was increased. The maximum enhancement of exchange aberrations was shown in the combined treatment with X-rays and OTC-3. Exchange aberration formation is thought to arise as a consequence of illegitimate reunion (misrejoining) of free ends from different DNA double strand breaks (dsbs) [15]. Therefore, it seems that OTC-3 induced DNA dsbs interact better way to the DNA dsbs induced by X-rays and therefore induced more delay in cell proliferation. Diorganotin(IV) complexes inhibit the DNA synthesis of spleen cells in mice [16] and decreases the proliferation of human B lymphocytes [17]. Owing to the structural similarity of these diorganotin(IV) complexes to cisplatin, it could be that these diorganotin(IV) complexes like cisplatin cross-linked to the N^7 position of two consecutive guanine molecules in DNA and distort the DNA double-helix. It has also been demonstrated that dimethyltin(IV) dichloride form adducts with adenine, 9-methyl adenine and adenosine [18]. Moreover, due to structurally advantages of the present diorganotin(IV) complexes, particularly increased bond-length of Sn–N bond, it could be inferred that these compounds could bind on DNA more easily.

In the attempt to elucidate whether these compounds might affect genes playing a role in G1/S phase transition, the expression of p53 and p21 (WAF1), mainly involved in response to DNA-damaging stress [19], were analyzed by immunoblotting. It is well known that nuclear phosphoprotein encoded by the tumour suppressor gene p53 is a crucial component of the cellular pathways that are invoked in response to DNA damage. In the present study, the level of p53-protein was raised after treatment with diorganotin(IV) complexes and the induction was maximum in the OTC-3 treated sample. The level of p21 was also enhanced equally and significantly in all the treated samples. Therefore, present data indicate that significant increase in p21 in unstimulated Go lymphocytes could be a factor responsible for diorganotin(IV)-induced cell cycle arrest.

The increase of exchange aberrations in the combined treatment is important since such aberrations carrying cells may die apoptotically [20]. To examine the pathway of antitumor activity of diorganotin(IV) compounds DNA fragmentation analysis were, therefore, carried out here in Dalton's lymphoma cells. The results indicated that both OTC-3 alone or in combination with X-rays triggered apoptosis in Dalton's lymphoma cells 24 h after the treatment. Stridh et al. [21] demonstrated that tributyltin and triphenyltin could kill target cells by triggering apoptosis in human Hut-78 and Jurkat T-lymphocyte cell lines by increasing caspase activity. The present apoptosis induction could be a p53-dependent signaling pathway since the level of p53 was found to be the most promising in OTC-3 treated cells in human lymphocytes. It has been reported that p53 can act as a trans-activator or repressor for a set of pro- or anti-apoptotic genes. Survivin is one of such

target genes [22] known as inhibitor of apoptosis protein, which plays a key role in the regulation of apoptosis and cell division [23]. We have demonstrated that the upregulated expression of p53 caused a significant down-regulated transcriptionally repression of Survivin in OTC-3 treated human lymphocytes. Therefore, it may be possible that cells may die apoptotically after inducing initial delay in cell cycle and thereby survivality of mouse bearing DL cells was increased significantly in X-rays and OTC-3 combined treatment.

The present anti-proliferative property of all these diorganotin(IV) complexes alone or in combination with X-rays could increase the survivality of mouse bearing Dalton's lymphoma cells. It has been observed that diphenyltin(IV) compound inhibited the tumor growth in mice bearing Ehrlich ascites tumor cells [24]. Earlier studies with these diorganotin(IV) complexes showed its potential to increase the survival of mice bearing DL and demonstrated its antiproliferative ability [9, 10]. The trypan blue dye exclusion assay in the previous study showed that OTC-3 induced cell death was highest in frequency than other two organotin(IV) compounds [9]. In this study better antiproliferative and apoptosis-inducing ability of OTC-3 made it a better compound having higher antitumor potentiality than OTC-1 and OTC-2. A quantitative structure–activity relationship explained that the tendency to obtain condensed R_nSn(IV)-DNA as a function of the lipophilicity increases in the series Me<Et<n-Bu [25]. Therefore, it seems that by better binding of OTC-3 with DNA, it induces higher DNA damage and delay in cell proliferation. In this study, all the three diorganotin(IV) complexes has been tested for genotoxicity since there is a regulatory requirement for such testing using a number of different end-points before new drugs are released [26]. However, the mechanism of action of OTC-3 could be different since in the previous study it was shown that the endogenous GSH level has no influence on OTC-3 action [9] and in the present study it was demonstrated that only OTC-3 could induce apoptotic cell death but not the OTC-1 and OTC-2.

Acknowledgements This work was supported partly by grants from the Council of Scientific and Industrial Research, New Delhi, India (Grant No. 9/347(118)/97/EMR-I) and partly by grants from the University Potential for Excellence Programme from University Grants Commission, New Delhi to AC and from the Department of Science and Technology, New Delhi, India (Grant No. SR/SI/IC-03/2005) to TSBB.

References

1. Pellerito L, Nagy L (2002) Organotin(IV)$^{n+}$ complexes formed with biologically active ligands: equilibrium and structural studies, and some biological aspects. Coord Chem Rev 224:111–150. doi:10.1016/S0010-8545(01)00399-X
2. Gielen M, Tiekink ERT (eds) (2005) Metallotherapeutic D and metal-based diagnostic agents: ^{50}SnTin compounds and their therapeutic potential. Wiley, Chichester, pp 421–439
3. Li Q, Yang P, Wang H, Guo M (1996) Diorganotin(IV) antitumor agent. (C2H5)2SnCl2 (phen)/nucleotides aqueous and solid-state coordination chemistry and its DNA binding studies. J Inorg Chem 15;64(3):181–195
4. Crowe AJ (1987) The chemotherapeutic properties of tin compounds. Drugs Future 12:255–275
5. Gielen M, Willem R, Bouhdid A, De Vos D, Kuiper CM, Veerman G et al (1995) In vitro antiproliferation effects, toxicity profiles in vivo in mice and antitumor activity in tumor bearing mice of five diorganotin compounds. In Vivo 9:59–64
6. Crowe AJ, Smith PJ, Cardin CJ, Parge HE, Smith FE (1984a) Possible predissociation of diorganotin dihalide complexes: relationship between antitumor activity and structure. Cancer Lett 24:45–58. doi:10.1016/0304-3835(84)90078-8
7. Crowe AJ, Smith PJ, Atassi G (1984b) Investigations into the antitumor activity of organotin compounds. 2. Diorganotin dihalide and dipseudohalide complexes. Inorg Chim Acta 93:179–184. doi:10.1016/S0020-1693(00)88160-8
8. Basu Baul S, Basu Baul TS, Rivarola E, Dakternieks D, Tiekink ERT, Syng-ai C et al (1998) Synthesis and characterization of diorganotin(IV) complexes of N-(2-Pyridylmethylene)-arylamines and Mutagenecity testing of $Et_2SnCl_2L^4$. Appl Organomet Chem 12:503–513. L^4=N-(2-Pyridylmethylene)-4-toluidine. doi:10.1002/(SICI)1099-0739(199807)12:7<503::AID-AOC746>3.0.CO;2-P
9. Koch B, BasuBaul TS, Chatterjee A (2008) Cell Proliferation inhibition and antitumor activity of novel alkyl series of diorganotin(IV) compounds. J Appl Toxicol 28:430–438. doi:10.1002/jat.1290
10. Syng-ai C, Basu Baul T, Chatterjee A (2002) Antiproliferative and cytotoxic effect of a novel organotin compound on mammalian cells both in vitro and in vivo. Mutat Res 513:49–59
11. Einhorn N, Trope C, Ridderhiem M, Boman K, Sorbe B, Cavallin-Stahl EA (2003) Systematic overview of radiation therapy effects in cervical cancer (cervix uteri). Acta Oncol (Madr) 42:546–556. doi:10.1080/02841860310014660
12. Goto K, Akematsu T, Shimagu H, Suigiyama T (1975) Simple differential Giemsa staining of sister chromatids after treatment with photosensitive dyes and exposure to light and the mechanism of staining. Chromosoma 53:223–230. doi:10.1007/BF00329173
13. McFee AF, Sayer AM, Salomaa SI, Lindholm C, Littlefield LG (1997) Methods for improving the yield and quality of metaphase preparations for FISH probing of human lymphocyte chromosomes. Environ Mol Mutagen 29:98. doi:10.1002/(SICI)1098-2280(1997)29:1<98::AID-EM13>3.0.CO;2-C
14. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD et al (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150:76–85. doi:10.1016/0003-2697(85)90442-7
15. Cornforth MN, Bedford JS (1993) Ionizing radiation damage and its early development in chromosomes. In: Lett JT, Sinclair WK (eds) Advances in Radiation Biology, Vol. 17: DNA Damage Caused by Radiation. Academic, London
16. Al-Imara L, Salaman MR, Sljivic VS, Poller RC (1993) Inhibition of mouse spleen cell activity by organotin compounds: effect of attachment to a maltose residue to the organotin group. Int J Immunopharmacol 15:287–291
17. De Santiago A, Aguilar-Santelises M (1999) Organotin compounds decrease in vitro survival, proliferation and differentiation of normal human B lymphocytes. Hum Exp Toxicol 18:619–624. doi:10.1191/096032799678839437
18. Pellerito L, Nagy L (2002) Organotin(IV)$^{n+}$ complexes formed with biologically active ligands: equilibrium and structural

studies, and some biological aspects. Coord Chem Rev 224:111–150. doi:10.1016/S0010-8545(01)00399-X
19. Rudoltz MS, Kao G, Blank KR, Muschel RJ, McKenna WG (1996) Molecular biology of the cell cycle: potential for therapeutic applications in radiation oncology. Semin Radiat Oncol 6:284–294. doi:10.1016/S1053-4296(96)80024-5
20. Bassi L, Carloni M, Meschini R, Fonti E, Palitti F (2003) X-irradiated human lymphocytes with unstable aberrations and their preferential elimination by p53/surviving-dependent apoptosis. Int J Radiat Biol 79:1–12. doi:10.1080/713864981
21. Stridh H, Orrenius S, Hampton MB (1999) Caspase involvement in the induction of apoptosis by the environmental toxicants tributyltin and triphenyltin. Toxicol Appl Pharmacol 156:141–146. doi:10.1006/taap.1999.8633
22. Hoffman WH, Biade S, Zilfou JT, Chen J, Murphy M (2002) Transcriptional repression of the anti-apoptotic surviving gene by wild type p53. J Biol Chem 277:3247–3257. doi:10.1074/jbc.M106643200
23. Deveraux QL, Reed JC (1999) IAP family proteins-suppressor of apoptosis. Genes Dev 13:1253–1262. doi:10.1101/gad.13.3.239
24. Cookson MR, Slamon ND, Pentreath VW (1998) Glutathione modifies the toxicity of triethyltin and trimethyltin in C6 glioma cells. Arch Toxicol 72(4):197–202. doi:10.1007/s002040050488
25. Rekker RF (1977) The hydrophobic fragmental constant. Elsevier, Amsterdam
26. Anon. Guidelines for the testing of Chemicals for Mutagenicity, Report on Health and Social Subjects, No. 35, Department of Health, HMSO, London, 1989