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Spindle-inhibiting effects of organotin compounds. II. Induction of chromosomal supercontraction by di- and tri-alkyl and -aryl compounds*

Klaus Gjervig Jensen,† Ole Andersen† and Mogens Rønne‡
†Department of Environmental Medicine and ‡ Winslow Institute of Human Anatomy, Odense University, Odense, Denmark

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Spindle-inhibiting effects of chemical compounds may be studied indirectly by quantitation of chromosomal contraction. The effects of the trimethyltin (TMT), dimethyltin (DMT), tributyltin (TBT), dibutyltin (DBT), triphenyltin (TPhT) and diphenyltin (DPht) moieties as the chloride on chromosomal contraction was studied by measurement of the average length of chromosome No. 1 from asynchronous cultures of human peripheral lymphocytes. TMT, TBT, TPhT and DPhT appear to be very strong inducers of chromosomal supercontraction, indicating that these compounds conceivably are spindle inhibitors, whilst DMT and DBT seem to be ineffective. The different effects of aryl versus alkyl and trivalent versus divalent organic substituents of tin on chromosome length may relate to different modes of action.

Keywords: Organotin, spindle inhibition, chromosome length, *in vitro*, human lymphocytes

INTRODUCTION

Organic tin compounds started to gain commercial significance in the 1950s, and a larger number of organic compounds of tin than of any other element are presently in commercial use. Industrially important tin compounds are especially those with methyl, butyl, octyl and phenyl groups as organic substituents, with chloride, fluoride, oxide, hydroxide, carboxylate or thiolate as the most commonly used anions.

Diorganotin compounds, as dimethyltin (DMT) and dibutyltin (DBT) chlorides, are used as heat and light stabilizers in PVC plastics; triorganotin compounds, as tributyltin (TBT) and triphenyltin (TPhT) chlorides, are used as pesticides because of their fungicidal and bactericidal properties, and both DBT and TBT chlorides are used in marine antifouling paints.[1–3] The organotin compound bis(tri-n-butyltin) oxide (TBTO) has been proposed as a molluscicide to control the snail vector of Schistosomae.[4,5]

High doses of TBTO (60 mg kg^{-1} bodyweight) induced a significant positive response in the micronucleus test in male mice,[6] indicating chromosome damage or malfunction of the mitotic apparatus. Using purified rat brain tubulin, Tan *et al.*[7] demonstrated that trialkyltin and triaryltin compounds inhibited tubulin polymerization and decreased the colchicine binding activity of tubulin *in vitro*. In addition, exposure of algae to organotin compounds was reported by Röderer[8] to disturb mitosis and cytokinesis and to induce formation of multinucleated cells and polyploid nuclei. Thus, organotin compounds are capable of inhibiting the function of the spindle.

Highly contracted chromosomes have been observed in several experimental studies with spindle inhibitors (see for example Östergreen[9]). Consequently, a statistical method developed for quantitating small differences in chromosome structure by chromosome length measurements[10,11] could be used to investigate the effects of well-known spindle-inhibiting compounds on the chromosome contraction processes.[12–18]

Also, we recently demonstrated that *in vitro* exposure of human lymphocytes to TMT chloride resulted in a statistically significant reduction in average chromosome length.[19] Using the same method of

* For paper I in this series see Ref. 19.

analysis, the present study reports the effects of *in vitro* exposure of human lymphocytes to TMT, DMT, TBT, DBT, TPhT, and DPhT as the chlorides on average chromosome length.

MATERIALS AND METHODS

Human peripheral blood was obtained from donors selected at random. Lymphocyte cultures (0.5 cm^3 blood in RPMI 1640 with 10% foetal calf serum; 50 $\mu g\ cm^{-3}$ gentamycin; 5 i.u. cm^{-3} heparin; 0.2 cm^3 PHA-M (Gibco); and 0.1 cm^3 Hepes buffer in a total volume of 10 cm^3 at a pH of approximately 7.25, were incubated in 5% carbon dioxide in air at 37.5°C for a total of 72 h. The concentrations of the organotin compounds varied between 10^{-3} mol dm^{-3} and 10^{-9} mol dm^{-3}. The exposure time was 24 h. Due to the lower water solubilities of some of the organotin compounds used, dimethyl sulphoxide or ethanol was used as solvent in most of the experiments. The more water-soluble tin compounds were solubilized in the growth medium. The possible effects of dimethyl sulphoxide and ethanol on chromosome length were therefore studied initially. The solvent (20 μL) was added to a culture flask to give a total volume of 10 cm^3 and a concentration of 0.2%. The control culture contained the same volume of growth medium.

After hypotonic treatment with 0.075 mol dm^{-3} potassium chloride and fixation with acetic acid–methanol (1:3), air-dried slides were prepared, stained in Giemsa and mounted with Eukitt. From 100 metaphases selected at random the length of one chromosome No. 1 was determined at intervals of 1 μm with a calibrated measuring eye-piece. Data from different cultures were compared using Kolmogorov–Smirnov's two-tailed test for two independent samples.[20] The significance limit chosen was $P = 0.01$. Since $N = 100$ in all samples, this limit equals a D-value of 0.23, where D is the maximal difference between the cumulative distributions.

RESULTS

Effects of organotin chloride compounds on chromosome length are summarized in Table 1. The final concentration of the solvents dimethyl sulphoxide and ethanol in the culture flask was 0.2%. The average chromosome length in control cultures with and without 0.2% dimethyl sulphoxide was 11.95 ± 2.15 μm and 11.85 ± 2.55 μm, respectively. Comparison of length distribution frequencies from the two independent samples gave a D-value as low as 0.06. For ethanol, the largest difference between controls with and without 0.2% ethanol added was seen in samples with average chromosome length of 11.74 ± 2.16 μm and 10.79 ± 2.36 μm, respectively, and comparison of samples as before gave a D-value of 0.14. This indicates that neither dimethyl sulphoxide nor ethanol had a measurable effect on the chromosome contraction processes at the concentration used.

Significant differences between test and control cultures (Table 1, $D \geq 0.23$) indicate increasing threshold values for induction of supercontraction as follows: TPhT (3×10^{-8} mol dm^{-3}) < TBT (10^{-7} mol dm^{-3}) < TMT (3×10^{-7} mol dm^{-3}) < DPhT (10^{-6} mol dm^{-3}). Exposure to DMT and DBT apparently did not affect chromosome length. While TMT gradually reduced the average chromosome length at increasing concentrations, the effect of TBT, TPhT and DPhT occurred with more distinct thresholds, below which no effect was observed and above which supercontraction was rapidly achieved.

Exposure to 3×10^{-4} mol dm^{-3} TMT resulted in maximum chromosome contraction and an exceptionally low average chromosome length of 2.73 μm (Table 1). In comparison, exposure to TBT, TPhT and DPhT at concentrations inducing maximum chromosome contraction (10^{-6} mol dm^{-3}, 3×10^{-7} mol dm^{-3} and 10^{-6} mol dm^{-3}) resulted in average chromosome lengths of 7.26, 7.54 and 8.68 μm, respectively. These concentrations were the highest allowing chromosome length measurements, as higher concentrations were cytotoxic.

As the hypotonic treatment partially disrupts the spindle, C-mitosis-like metaphases occasionally occur on slides from control cultures. A quantitative evaluation of induction of C-mitosis therefore requires a preparation technique that excludes chromosome length measurement. Accordingly, such data are not given in the Table. However, slides from the different cultures showed very clear differences in frequencies of C-mitosis-like metaphases. Less that 10% 'C-mitoses' were found in control cultures while in cultures exposed to those organic tin compounds that were powerful inhibitors of chromosomal supercontraction, almost all metphases appeared like

Table 1 Effects of organotin compounds on average chromosome length[a]

Concn mol dm^{-3} $X \pm SD(\mu m)$[d]						
Compounds:[b] Solvent:[c]	TMT DMSO	DMT Medium	TBT Ethanol	DBT DMSO	TPhT DMSO	DPhT Ethanol
Control	11.42 ± 2.13	10.63 ± 1.89	11.14 ± 2.16	11.02 ± 1.83	10.65 ± 1.84	10.84 ± 1.96
1×10^{-9}	10.99 ± 1.86 (0.12)	11.05 ± 1.87 (0.18)		11.54 ± 2.00 (0.11)		10.93 ± 2.01 (0.06)
3×10^{-9}			10.92 ± 2.35 (0.06)	11.43 ± 2.00 (0.08)	11.03 ± 2.11 (0.08)	
1×10^{-8}	11.19 ± 1.61 (0.09)	11.40 ± 2.59 (0.22)	10.52 ± 2.33 (0.14)	11.00 ± 1.96 (0.04)	9.71 ± 1.92 (0.21)	10.97 ± 2.00 (0.06)
3×10^{-8}			10.57 ± 2.27 (0.14)	11.73 ± 1.77 (0.16)	9.86 ± 1.92 (0.21)	
1×10^{-7}	10.80 ± 1.91 (0.15)	10.80 ± 2.10 (0.06)	10.85 ± 2.68 (0.09)	10.72 ± 2.13 (0.15)	8.50 ± 1.71 (0.48*)	10.95 ± 2.35 (0.04)
3×10^{-7}	10.29 ± 2.38 (0.21)	10.62 ± 1.92 (0.06)	8.88 ± 2.53 (0.39*)	10.56 ± 1.93 (0.12)	7.54 ± 1.63 (0.62*)	11.21 ± 2.19 (0.10)
1×10^{-6}	9.92 ± 2.03 (0.36*)	10.31 ± 2.21 (0.10)	7.26 ± 2.76 (0.63*)	†	†	10.70 ± 2.51 (0.07)
3×10^{-6}	8.86 ± 1.91 (0.50*)	11.26 ± 2.28 (0.13)	†	†	†	8.68 ± 3.01 (0.39*)
1×10^{-5}	8.52 ± 1.76 (0.56*)	10.81 ± 2.49 (0.14)	†	†	†	†
3×10^{-5}	6.88 ± 1.80 (0.76*)	11.14 ± 2.17 (0.11)	†	†	†	†
1×10^{-4}	4.29 ± 1.47 (0.94*)	10.64 ± 3.39 (0.16)	†	†	†	†
3×10^{-4}	2.73 ± 0.49 (1.00*)	†	†	†	†	†
1×10^{-3}	†	†	†	†	†	†

[a]The exposure time was 24 h. [b]As chlorides; abbreviations in text. [c]Abbreviation: DMSO, dimethyl sulphoxide. [d]X=measured length of chromosome. *D*-values are in parentheses. *Significant at 0.01% level. †No result due to the toxicity of the treatment.

C-mitoses at the highest concentration. Conversely, only about 20% of metaphases from the cultures exposed to 10^{-4} mol dm^{-3} DMT were C-mitosis-like, indicating that this compound is a rather weak spindle inhibitor.

DISCUSSION

In experiments with human lymphocytes where necessary components are insoluble in the culture medium, the use of DMSO or ethanol as solvent has been suggested, provided that the final volume of the solvent in the culture is less than 1% of the total.[21] Dimethyl suphoxide was reported to accelerate tubulin aggregation and stabilize preformed aggregates *in vitro*, but such effects have not been observed at concentrations as low as the 0.2% used in this study.[22] As demonstrated above, neither of the solvents significantly affected the chromosome contraction process at this concentration.

In this study, TMT and TBT definitely induced chromosomal supercontraction while DMT and DBT had no measurable effect on the chromosome length distribution. This could indicate a requirement for tri-substitution for alkyltin compounds to achieve a potency for the induction of supercontraction. However, both DPhT and TPhT were clearly capable of inducing supercontraction, indicating a possible difference between alkyltin and aryltin compounds, which would merit further investigation.

The kinetics of induction of supercontraction by TMT differs from those of TBT, TPhT and DPhT.

These latter had sharp thresholds, below which no effect was observed and above which complete supercontraction were rapidly achieved. A similar effect was earlier observed for induction of chromosomal supercontraction by colchicine.[15,18] Conversely, gradually reduced average chromosomal length was observed at increasing concentrations of TMT.

Compared with the reported threshold concentrations of about 10^{-6} mol dm^{-3} for supercontraction induced by triethyl-lead chloride[18] and about 3×10^{-7} mol dm^{-3} for supercontraction induced by colchicine,[15] TMT, TBT, TPhT and DPhT are potent inducers of supercontraction with threshold values from 3×10^{-8} mol dm^{-3} TPhT to 10^{-6} mol dm^{-3} DPhT. Assuming a causal relation between the capability for induction of supercontraction and a spindle-inhibiting potential, these threshold values may be compared with reported threshold concentrations between 10^{-6} mol dm^{-3} and 10^{-7} mol dm^{-3} for induction of C-mitosis in allium roots by colchicine and organic compounds of mercury and lead.[23,24]

In this study, the spindle-inhibiting potency of organotin compounds was assessed indirectly by chromosome length measurements and demonstrated more directly by the presence of typical C-mitoses on slides from cultures exposed to organotin compounds. Assuming that the molecular mechanism of action is similar to that of colchicine, *in vitro* exposure of tubulin to organotin compounds would be expected to inhibit the colchicine-binding activity. Accordingly, TBT and TPhT and certain other triorganotin compounds were found to reduce the colchicine-binding activity of tubulin *in vitro*, while TMT led to an unexpected increase in colchicine binding,[7] indicating a different mode of action. Measurement of tubulin polymerization *in vitro* by viscometry indicated that all triorganotin compounds induced identical decreases in polymerization, except for TMT which was less effective.[7]

The results presented here indicate that TPhT, DPhT and TBT possess spindle-inhibiting properties that are related to the colchichine mode of action towards the spindle apparatus, while TMT also has spindle-inhibiting potency, but presumably acts by a different mechanism.

Organotin compounds are toxic towards a variety of organs, e.g. the central nervous system, the immune system and the liver. On a cellular basis, toxicity has been described as a suppression of the energy state and inhibition of macromolecular synthesis and cell proliferation,[25] while spindle-inhibiting effects of the compounds have received relatively little attention.

In accordance with the observed disturbances of mitosis and cytokinesis observed in algae,[8] the induction of micronuclei in mice[6] and the inhibition of tubulin polymerization and colcohicine binding *in vitro*,[8] this paper demonstrates that organotin compounds are also able to induce chromosomal supercontraction, indicating a spindle-inhibiting potency. This is further supported by the observation of high frequency of C-mitoses on slides from organotin-treated cultures. At concentrations around the threshold, organotin compounds may give rise to partial spindle inhibition leading to a high probability of non-disjunction.

Some spindle-disturbing agents have been demonstrated to increase the frequency of aneuploidy *in vitro* and *in vivo*.[26–28] Thus, exposure to organotin compounds could conceivably lead to an increased risk for induction of aneuploidy.

Aneuploidy due to meiotic non-disjunction is the cause of several severe genetic diseases as well as a significant fraction of foetal losses and early infant mortality.[29] Mitotic non-disjunction during early embryogenesis may cause the same effects. Induction of aneuploidy in mannalian cells by organotin compounds has not yet been demonstrated directly. Accordingly, further investigations of the effects of these industrially important compounds on the spindle apparatus and the mitotic process are of considerable interest.

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