

Disturbed microtubule function and induction of micronuclei by chelate complexes of mercury(II)

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Abstract

Interactions of mercury(II) with the microtubule network of cells may lead to genotoxicity. Complexation of mercury(II) with EDTA is currently being discussed for its employment in detoxification processes of polluted sites. This prompted us to re-evaluate the effects of such complexing agents on certain aspects of mercury toxicity, by examining the influences of mercury(II) complexes on tubulin assembly and kinesin-driven motility of microtubules. The genotoxic effects were studied using the micronucleus assay in V79 Chinese hamster fibroblasts. Mercury(II) complexes with EDTA and related chelators interfered dose-dependently with tubulin assembly and microtubule motility *in vitro*. The no-effect-concentration for assembly inhibition was 1 μM of complexed Hg(II), and for inhibition of motility it was 0.05 μM , respectively. These findings are supported on the genotoxicity level by the results of the micronucleus assay, with micronuclei being induced dose-dependently starting at concentrations of about 0.05 μM of complexed Hg(II). Generally, the no-effect-concentrations for complexed mercury(II) found in the cell-free systems and in cellular assays (including the micronucleus test) were identical with or similar to results for mercury tested in the absence of chelators. This indicates that mercury(II) has a much higher affinity to sulfhydryls of cytoskeletal proteins than to this type of complexing agents. Therefore, the suitability of EDTA and related compounds for remediation of environmental mercury contamination or for other detoxification purposes involving mercury has to be questioned.

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1. Introduction

Mercury is an important environmental pollutant of surface water, ground water and soil [1,2], and a means of remediation of polluted sites are a major point of present discussion. Very recently, the possibilities of removal of mercury from soils using electrokinetics have been explored, and disodium ethylenediaminetetraacetate (Na₂-EDTA) has been addressed as complexing agent of greatest potential for this purpose [3]. This leads to the question of the toxicological behaviour of mercury complexes, as mercury is a chromosomal genotoxicant that elicits formation of micronuclei in mammalian cells.

This was the starting point to re-evaluate the effect of complexing agents on the genotoxicity of mercury(II), and of associated toxicological mechanisms. It extends previous studies on indirect genotoxicity and disturbed microtubule function caused by inorganic lead(II) and mercury(II) [4,5].

Inorganic mercury(II) is known for strong interactions with functional groups of proteins, especially with sulphhydryl groups [6,7]. Cytoskeletal proteins like tubulin or kinesin, which are basic constituents of eukaryotic cells, are involved in processes of cell movement, cell division and chromosomal segregation (for reviews see [8,9]). The force generating microtubule–kinesin system can be re-constituted *in vitro*, to elucidate effector activities on both microtubule structure and kinesin function.

Tubulin and kinesin are important targets for mercury(II), and the powerful inhibitory influence of Hg(II) on tubulin assembly has been extensively studied [4,5,7,10–13]. As far as mercury(II) complexes are concerned, Duhr et al. [13] have specifically looked at the combinations of mercury(II) with EDTA and EGTA (ethyleneglycol bis(2-amino-ethyl)-tetraacetic acid), to examine differential influences on microtubule formation. According to these authors, a target for free mercury(II) ions may be the tubulin dimer with its free sulphhydryl groups, whereas HgEDTA or HgEGTA complexes tend to block the interaction between tubulin and the energy donor GTP.

Our intention was to advance the concept of interaction of Hg(II) complexes with cellular motor protein functions, as this might necessitate to re-assess current views of Hg(II) complexation as a means of Hg detoxification. The aim was also to further elucidate the way

by which mercury(II) exerts its genotoxic potential on the chromosome level.

2. Materials and methods

2.1. Chemicals

Mercury(II) chloride [CAS No. 7487-94-7] was purchased from Sigma–Aldrich (Taufkirchen, Germany). Mercury chloride was first dissolved in water before it was added to the buffer solution or the cell culture medium containing the complexing agent. The complexing reagents EGTA [67-42-5], EDTA [60-00-4], and DTT [27565-41-9] were products of Roth (Karlsruhe, Germany), and NTA [139-13-9] and L-cysteine [52-90-4] came from Merck (Darmstadt, Germany). All other chemicals, biochemical and cell culture reagents were from the same sources as given previously [5].

2.2. Microtubule assembly, turbidity assay

Microtubule protein (MTP; tubulin containing microtubule associated proteins, MAPs) was isolated from porcine brain by two cycles of temperature-dependent assembly–disassembly [14], with modifications described in [5].

Microtubule assembly was recorded using a spectral photometer [15], with the same adjustments as published in [5]. Twenty minutes after inducing MTP polymerisation at 37 °C, the turbidity curve has reached a plateau level (assembly–disassembly steady state). The corresponding absorbance was taken as a reference value to quantify the effects of mercury and mercury complexes. To check the reversibility of microtubule assembly, the measurement was continued for additional 20 min while decreasing the temperature to 4 °C.

2.3. Microtubule disassembly, video-enhanced microscopic assay

Microtubules were formed from 0.8 mg/ml MTP using the “assembly buffer” [5] (the complexing agent included) by 20 min incubation at 37 °C and stabilised with paclitaxel (10 μM final concentration). This suspension was 10-fold diluted in water containing pacli-

taxel (10 μM) and HgCl_2 (5 μM), and transferred onto glass slides. By differential interference contrast microscopy (see 2.5), the disassembly of individual microtubules was followed over 15 min.

2.4. Transmission electron microscopy

Negative staining of microtubules formed during the turbidity assay in the presence of mercury chloride (at IC_{50}) and EGTA was performed using 1% uranyl acetate (from Merck, Darmstadt) in water [16]. The sample structures were visualized with a Zeiss EM 902 A transmission electron microscope.

2.5. Microtubule gliding assay

Microtubule protein (MTP) was isolated from porcine brain [14], and purified from MAPs by phosphocellulose ion exchange chromatography [17]. Kinesin was purified from porcine brain homogenates by a combined procedure of ion exchange chromatography, microtubule affinity-binding in the presence of tripolyphosphate, and gel filtration [18].

The microtubule gliding assay was performed according to a basic protocol given by Böhm et al. [19], with the following modifications: at first, kinesin (5 μl , 1 mg/ml) was allowed to attach onto an 18 mm \times 18 mm area of a glass slide, then non-bound kinesin was removed by washing with 100 mM imidazole buffer (pH 6.8) supplemented with 0.5 mM $\text{Mg}(\text{NO}_3)_2$. Thereafter, 10 μl of a suspension of paclitaxel-stabilised microtubules (40 $\mu\text{g}/\text{ml}$ tubulin) in the same buffer containing 0.5 mM Na_2ATP and mercury(II) chloride plus the complexing agent (EGTA, 1 mM) were added before the area was covered by a coverslip. Then the gliding activity was monitored by video-enhanced differential interference contrast microscopy (principles described by Weiss and Maile [20]), using an Axiophot microscope (Zeiss) equipped with the image processing system Argus 20 (Hamamatsu). Gliding velocities were directly measured using the speed function of the Argus image processor.

2.6. Cells

V79 hamster lung fibroblasts were used in the cytotoxicity assay, the micronucleus test, and in immunofluorescence studies. These fibroblasts contain a set of 22

chromosomes and have a population doubling time of 12 h. The cells were cultured in sterile flasks or on sterile slides in DMEM containing 10% FCS (incubation at 37 °C, 5% CO_2). All cells used in our assays were tested negative for mycoplasmas.

2.7. Cytotoxicity and micronucleus assays

The cytotoxicity of mercury chloride and its complexes was determined in V79 hamster lung fibroblast cells by means of the neutral red assay [21], performed as described previously in more detail [22]. Briefly, V79 cells were plated in medium in 96-well tissue-culture plates, and allowed to grow for 24 h at 37 °C. Then, mercury(II) chloride and/or EGTA were added to culture medium at various concentrations given in Fig. 5. After 18 h, the medium was replaced by a fresh one containing only neutral red (NR, 50 $\mu\text{g}/\text{ml}$), and incubation was continued for 3 h at 37 °C. The cells were then washed five times with PBS, and treated with 0.2 ml fixative (glacial acetic acid:water:ethanol, 1:49:50) per well. After 20 min of shaking (600 rpm) to bring the dye into solution, the NR absorbance was measured with a plate photometer (340 ATC, SLT) at 540 nm. The results were used to define a suitable range of concentrations (below and near cytotoxicity) for the genotoxicity assay with V79 cells.

The micronucleus (MN) assay was performed according to Matsuoka et al. [23], with modifications described in [22]. The cells were cultured in DMEM medium containing 10% FCS, and kept in an atmosphere with a content of 5% CO_2 (in open flasks or flasks with an appropriate filter enabling gas exchange). Briefly, V79 hamster lung fibroblasts were incubated for 1.5 cell cycles (i.e., 18 h) with the test compounds at concentrations given in Table 1 and Fig. 6. The cells were fixed, stained and a minimum of 8000 up to 18,000 cells were scored for each mercury concentration group. For each concentration, this afforded at least two parallel sets of experiments. The mean values and standard deviation were finally calculated for the total number of cells assessed per group.

2.8. Immunofluorescence studies

These experiments using V79 cells were performed according to procedures previously described in detail [5].

Table 1
Concentration-dependent induction of micronuclei in V79 cells by mercury chloride alone and in the presence of EGTA

Treatment (μM)		Cells scored	MN/1000 cells Means \pm S.D.	Remark
HgCl ₂	EGTA			
Series A				
0	0	16,000	4.4 \pm 0.7	Control A
0.005	0	12,000	5.1 \pm 0.6	n.s.
0.01	0	16,000	5.8 \pm 0.9	*
0.03	0	12,000	8.3 \pm 1.0	**
0.05	0	16,000	10.6 \pm 1.0	**
0.1	0	16,000	13.3 \pm 1.6	**
0.5	0	16,000	12.4 \pm 1.3	**
1.0	0	16,000	10.9 \pm 1.1	**
5.0	0	16,000	10.5 \pm 1.0	**
10.0	0	16,000	9.9 \pm 1.1	**
Series B				
0	0	16,000	5.3 \pm 1.0	Background
0	50	17,000	6.3 \pm 1.7	Control B
0.01	50	18,000	6.2 \pm 3.3 ^a	n.s.
0.05	50	18,000	7.7 \pm 2.2 ^b	n.s.
0.1	50	18,000	9.3 \pm 2.1 ^b	**
0.5	50	8,000	11.6 \pm 2.7 ^c	**
1.0	50	18,000	11.4 \pm 2.3 ^c	**
10.0	50	18,000	10.8 \pm 2.6 ^c	**

Due to the large number of groups, these experiments were conducted sequentially (series A and B) involving each the appropriate controls. n.s.: treatment group not significantly different from the concurrent control (A or B); *: different from control at $P < 0.1$; **: different from control at $P < 0.01$.

^a Weak or no genotoxicity and not sign. different between Series A and B.

^b Genotoxicity lower in Series B than in Series A.

^c Genotoxicity comparable in Series A and B.

3. Results

3.1. Turbidity assay

The turbidity assay is based on the observation that—under defined conditions—tubulin assembles and disassembles depending on the ambient temperature. Using MTP in a cell-free environment, physiological temperatures lead to polymerisation of the tubulin dimers and thus to microtubule formation. This process is reversible at low temperature.

Microtubule assembly and disassembly were observed spectrophotometrically during temperature cycles (from 4 °C to 37 °C and back to 4 °C). The tur-

bidity increases due to the formation of microtubules. If tubulin assembly is partly inhibited, e.g. by HgCl₂, the turbidity at 37 °C is reduced compared to the control sample. At a certain concentration, the assembly is completely inhibited (no rise in turbidity). Denaturation of tubulin with HgCl₂ followed by aggregation and precipitation may occur at higher concentrations and is reflected by a change in curve shape during the period of warming, compared to the control sample. A rise in absorption resulting from denaturation is not completely reversible, resulting in higher absorbances at 4 °C compared to control samples.

As already demonstrated [4,5], mercury(II) chloride affects tubulin assembly in a dose-dependent manner. Hg(II) inhibits microtubule assembly at concentrations above 1 μM . The inhibition is complete at about 10 μM . In this range the polymerisation is fully (up to 6 μM) or partially (~6–10 μM) reversible. Higher concentrations of mercury(II) cause the formation of protein–mercury aggregates. As it has been demonstrated for lead [4], the inhibition is independent of the anion used (chloride or nitrate). The no-observed-effect-concentration for inhibition of microtubule assembly inhibition at 1 mg/ml MTP is 1 μM mercury(II), the IC₅₀ is 5.8 μM .

Comparable effects are visible for the complexes of mercury(II) with nitrilotriacetic acid (NTA), EDTA and EGTA (Fig. 1). The no-observed-effect-concentration and IC₅₀ of mercury(II) are not significantly changed upon addition of these complexing agents. Hg²⁺ ions in presence of these chelators of the “amino acetic acid” type obviously exhibit the same dose–response relation as without the chelators.

On the other hand, typical representatives of the “sulfhydryl” type of complexing reagents, like L-cysteine or dithiothreitol (DTT), suppress the inhibition of tubulin assembly caused by 10 μM HgCl₂. This effect can even be detected, when the sulfhydryl compound is added after 20 min of pre-incubation of tubulin with Hg(II) (Fig. 2). Comparable results are seen in incubations, where NTA, EDTA or EGTA are added to the assembly mixture in addition to DTT (*data not shown*).

3.2. Transmission electron microscopy

The in vitro formation of microtubules and the possible formation of aberrant structures were also inves-

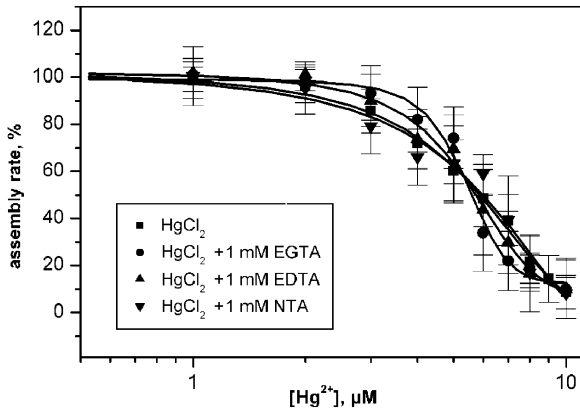


Fig. 1. Dose–response–relationships of inhibition of tubulin assembly by mercury(II) complexes of the “amino acetic acid” type compared to Hg(II) alone. Relative absorbance of the tubulin assembly mixture after incubation of tubulin with various concentrations of mercury(II) chloride and 1 mM of NTA, EDTA and EGTA (20 min at 37 °C). Each resulting point represents a mean of at least six test results, as percentage relative to control.

tigated by means of electron microscopy. As aberrant tubulin assemblies are usually due to disturbed lateral interaction of tubulin–dimers and/or protofilaments, inhibition of tubulin assembly not accompanied by morphological modifications of microtubules is likely a result of blocking the longitudinal tubulin–dimer association.

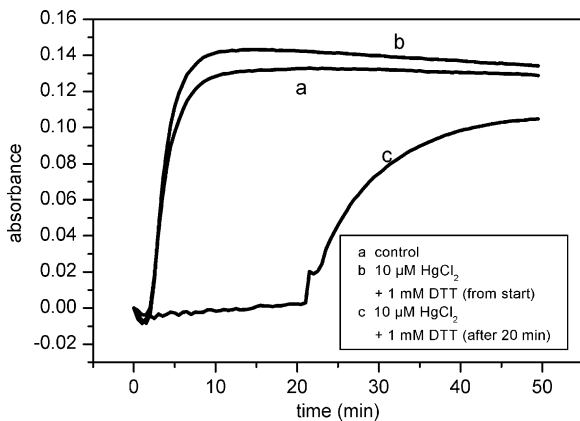
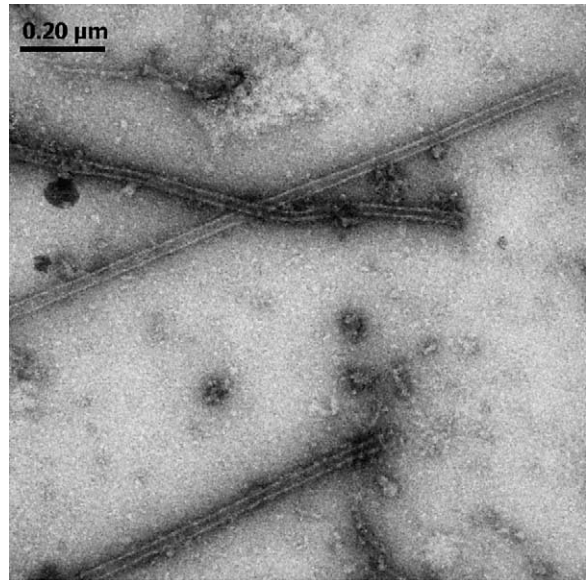
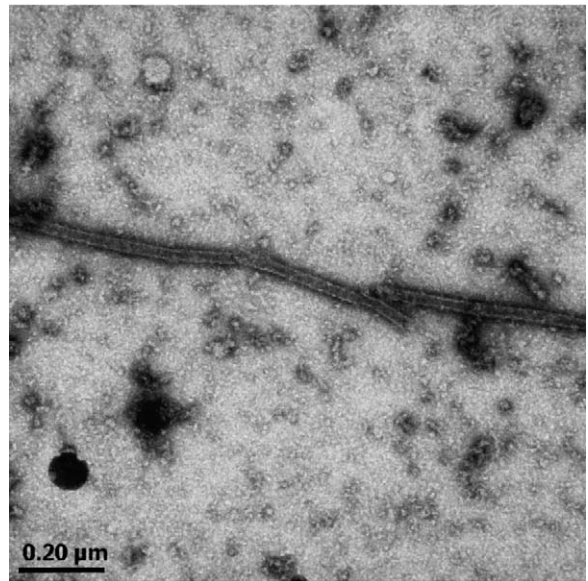


Fig. 2. Tubulin assembly assay with 10 µM HgCl₂ in the absence or presence of DTT (1 mM). Turbidity curves are shown without addition of DTT and HgCl₂, with DTT added prior to HgCl₂ or after 20 min of incubation with HgCl₂ at 37 °C.



(a)



(b)

Fig. 3. Electron micrographs of microtubules: (a) control, (b) microtubules incubated with 3 µM HgCl₂ and 1 mM EGTA.

Microtubules incubated in “assembly buffer” containing 3 µM mercury(II) and 1 mM EGTA show no significant structural deviations as compared to the control (Fig. 3). This means that Hg(II) in complex with EGTA at concentrations partly inhibiting tubulin as-

sembly does not cause the formation of aberrant microtubule structures.

3.3. Microtubule disassembly assay

Within cells, microtubules are attached with their minus ends to microtubule-organizing centres (MTOC), stabilising them against disassembly. To induce a fast disassembly of microtubules, special agents (e.g., katanin [24], endotoxin [25]) were shown to cleave them into numerous short pieces thereby increasing the number of free ends from which dimers can be detached. In this way, the efficiency of disassembly is improved.

As the turbidity assay measures only the overall assembly–disassembly and is not able to visualize fragmentation processes, disassembly of individual microtubules in the presence of the mercury(II) chloride–EGTA complex was followed by video-enhanced interference contrast microscopy. The images recorded for incubations with HgCl_2 ($5 \mu\text{M}$) plus EGTA (1 mM) show that the microtubules were only shortened, as in the case of HgCl_2 ($5 \mu\text{M}$) alone [5]. This indicates that mercury(II) induced disassembly is realized by endwise dimer release and not by fragmentation.

3.4. Microtubule gliding assay

Effects of the mercury(II) complex with EGTA on motility functions of the cytoskeleton were studied using the motor protein kinesin. The gliding assay mimics intracellular movement and transport processes in a cell-free system, examining the gliding of paclitaxel-stabilised microtubules across a kinesin-coated surface.

Fig. 4 demonstrates the concentration-dependent effect of mercury(II) chloride with or without EGTA on microtubule gliding. The gliding velocity is reduced above $0.05 \mu\text{M}$, and complete inhibition of motility is reached at about $1 \mu\text{M}$. The curves in the presence or absence of EGTA appear almost identical.

3.5. Cytotoxicity assay

In V79 cells, cytotoxicity measured as reduction of neutral red uptake for HgCl_2 , $\text{HgCl}_2 + 50 \mu\text{M}$ EGTA or $\text{HgCl}_2 + 100 \mu\text{M}$ EGTA starts at concentrations above

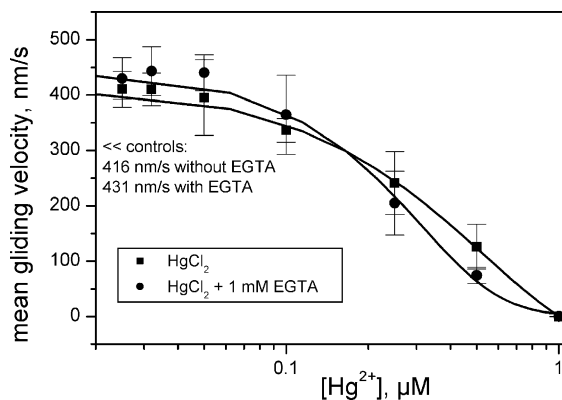


Fig. 4. Concentration-dependent inhibition of microtubule motility by mercury chloride in the absence or presence of EGTA. Each data point represents the mean of 15 measurements.

$17 \mu\text{M}$ HgCl_2 (Fig. 5). All curves have nearly the same progression, independent of EGTA ($50 \mu\text{M}$ or $100 \mu\text{M}$) being added or not. Between 17 and $20 \mu\text{M}$ HgCl_2 a 50% reduction of neutral red uptake was observed for both EGTA concentrations. For HgCl_2 alone, a 50% reduction of uptake was noted between 20 and $25 \mu\text{M}$ HgCl_2 , an LC_{50} value in accordance with previous data [4,5]. Additional experiments, at a constant concentration of $20 \mu\text{M}$ HgCl_2 and varying EGTA concentrations (0 – $200 \mu\text{M}$), showed also no significant influence of EGTA concentrations on the cytotoxicity of mercury (data not shown). EGTA (0 – $200 \mu\text{M}$) alone exerts no

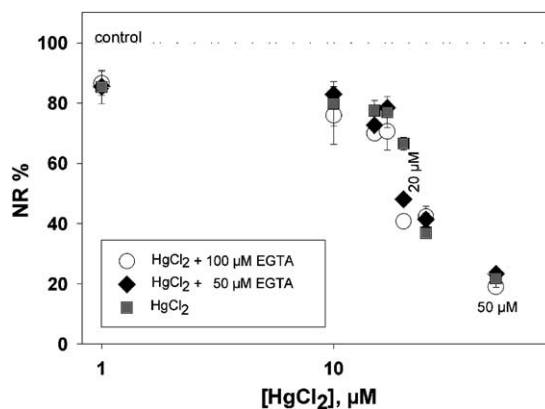


Fig. 5. Cytotoxicity of mercury chloride determined by uptake of neutral red in V79 cells (% of controls). Cells were treated for 18 h in the absence or presence of EGTA. Each data point represents the mean of 8 measurements.

cytotoxicity, as demonstrated in a neutral red assay. Moreover, controls with 1 mM EGTA alone had no negative influence on cell growth and the appearance of the microtubule cytoskeleton.

3.6. Micronucleus assay

Interactions of chemicals with cytoskeletal macromolecules are revealed by the micronucleus (MN) assay. Aneugenic compounds, causing spindle or kinetochore damage, lead to the formation of MN containing complete chromosomes. Clastogens may induce structural chromosome breaks.

Mercury(II) chloride, in the presence or absence of 50 μ M EGTA, induced a concentration-dependent increase in the number of micronuclei in V79 cells (Table 1). For both conditions, studied in sequential experiments, we observed an increase in micronuclei counts at mercury concentrations of about 0.05 μ M. Micronucleus (MN) rates increased with rising HgCl_2 concentrations, up to 0.1 μ M HgCl_2 or 0.5 μ M HgCl_2 ; at higher mercury(II) concentrations there is a slight decrease in micronuclei. In experiments with EGTA (50 μ M) rather similar MN rates were observed, although the standard deviations in the presence of EGTA were higher than in the experiments in the absence of EGTA. Nevertheless, the potencies of mercury(II) and Hg(II)/EGTA appear comparable. To confirm this, additional experiments were carried out at two HgCl_2 concentrations which induce a high rate of micronuclei (0.05 and 0.1 mM), alone and in combination with EGTA or DTT (50 μ M each). The results are shown in Fig. 6. The complexing agents, present at 500- to 1000-fold higher molar concentrations than mercury(II), did not affect its genotoxicity.

3.7. Immunofluorescence studies

The biochemical effects of mercury(II) complexed with EGTA were also studied by immunofluorescence staining of the microtubule cytoskeleton in V79 cells. Concentration-dependent disruptions of the network of microtubules were visualized by labelling with β -tubulin antibodies conjugated with Cy3 as a fluorophore.

With EGTA (1 mM) and up to 100 μ M Hg(II) there was no effect on the microtubule network in V79 cells. At a concentration of 150 μ M HgCl_2 , the network was

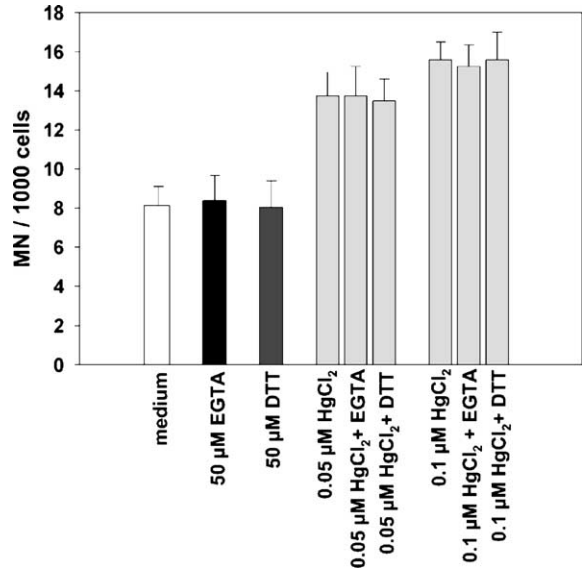


Fig. 6. Induction of micronuclei in V79 cells by mercury chloride (0.05 and 0.1 μ M) in the presence or absence of EGTA (50 μ M) or DTT (50 μ M). The frequency of MN is given as means \pm S.D. from evaluations of 8,000 cells per group. Also depicted is the background rate of micronuclei in untreated cells (medium) and in controls exposed to EGTA or DTT. All treatment groups were significantly different ($P < 0.01$) from the three controls; groups treated with mercury chloride alone did not differ from those with DTT or EGTA in the medium ($P > 0.5$ by *t*-test).

destroyed in some cells, whereas other cells remained intact. At 1 mM EGTA and 200 μ M mercury(II), all cells lost their microtubule cytoskeleton (*pictures not shown*). Thus, concentrations of mercury(II) affecting the integrity of microtubules were comparable to those found previously, in absence of EGTA [5].

4. Discussion

Because of a novel potential environmental impact [3], we investigated the interference of mercury(II) with complexing agents, especially of the “amino acetic acid” type, in terms of the functionality of the microtubule cytoskeleton. Interactions of mercury with these cellular structures are considered to be a basis for the chromosomal genotoxicity of mercury [4,5].

In this study, closer examinations were performed regarding the complex of Hg(II) with EGTA, including a determination of interactions with the motor protein

kinesin and with tubulin. Interestingly, this complex exerted virtually the same effects as mercury(II) alone, not only in all cell-free assays employed, but also on the cellular level as determined by the micronucleus assay and by cytotoxicity data in V79 cells.

Mercury(II) caused a concentration-dependent inhibition of kinesin-mediated microtubule motility in a cell-free assay, which became apparent at a concentration as low as 0.1 μM , while complete inhibition of motility was reached at 1 μM . Dose–response curves were alike in the presence or absence of EGTA (Fig. 4). These data point to kinesin as an interesting new target protein for cellular effects of mercury ions.

The chelators tested in the tubulin assembly assay together with mercury(II) can be divided into two categories:

The compounds with “amino acetic acid”-like structures (NTA, EDTA, EGTA), together with Hg^{2+} , lead to similar results as observed for free Hg^{2+} (Fig. 1). A potentiated inhibition of tubulin assembly, as proposed by Duhr et al. [13], could not be verified. Instead, our findings are in agreement with an earlier report [10], that EGTA does not affect the inhibition of tubulin assembly by mercury(II).

On the other hand, the sulfhydryl chelator DTT, once added at sufficient concentrations, completely suppressed the interaction between mercury and tubulin *in vitro* (Fig. 2). This was also found for L-cysteine, although the stability constant for the mercury complex of this amino acid ($\log K = 14$ [26]) is markedly lower than that for EDTA or EGTA ($\log K = 22$ or 23, respectively [26]).

It is generally accepted that binding of free mercury(II) to sulfhydryl groups in tubulin is critical for inhibition of its polymerisation to microtubules [6,7]. A total of 20 cysteine residues are contained in one single tubulin molecule [27]. An obvious conclusion to be drawn from the present results is that the affinity of mercury(II) to the sulfhydryl functions in tubulin is much higher than that to the O- and N-centres of EDTA and related chelating agents. Regardless of the very high stability constants of these complexes, tubulin seems to be capable of withdrawing mercury from these, and this finally results in a mercury-blocked tubulin activity with no potential for assembly, like it is found with free Hg^{2+} .

Much in contrast, the sulfhydryl reagents DTT and L-cysteine protect tubulin against the effects of mer-

cury(II) ions in the cell-free assay; even after 20 min of incubation of a tubulin assembly mixture containing Hg^{2+} , the blocked assembly process can be initiated by adding a sulfhydryl reagent, indicating that mercury immediately changes its reaction partner. These findings also provide evidence that the inactivation of tubulin by mercury(II) is not accompanied by protein denaturation, i.e., it is a reversible process. Furthermore, we found that, if a sulfhydryl reagent is added to the assembly mixture with mercury(II) after an “amino acetic acid type” compound, the influence of the sulfhydryl reagent is always dominating, regardless of their individual stability constants for mercury. Comparable results were reported by Keates and Yott [11], for 2-mercaptoethanol and Hg/EGTA.

It should be noted that one needs to be cautious in extrapolating findings from cell-free assays to cellular systems; thus, the thiol reagent DTT did not protect V79 cells against genotoxic effects elicited by mercury(II) concentrations much (500 to 1000-fold) lower than those of DTT in the culture medium (Fig. 6). How mercury(II) is taken up by cells is not yet clear. Some studies [28] suggest that conjugation of Hg^{2+} with L-cysteine promotes the transport of low concentrations of mercury, whereas an excess of glutathione (a common component in culture media) may interfere with uptake in membrane vesicles. Other studies [29] provide evidence that Hg^{2+} transport occurs mainly by diffusion and to a smaller extent by active transport.

Although we found no evidence that the mercury(II) complexes are more toxic than free Hg^{2+} once they have reached their protein targets, they could render the Hg^{2+} ion more readily available to target sites. This questions chelation with EDTA as a means of therapy after mercury intoxications [30]. In animals (toad), EDTA could not be used as an antidote for Hg(II) because of the inherent toxicity of the complex [31]. Furthermore, our new data about the chromosomal genotoxicity of mercury(II) complexes indicate similar potencies of Hg^{2+} , in free and complexed form (Table 1 and Fig. 6).

With regard to environmental toxicology, complexing agents of the “EDTA type” are very common additives in detergents and water softeners, and are also contained in foodstuff and medicines. This widespread use might lead to contaminations of surface and ground waters. EDTA and related compounds have also been

claimed to re-mobilise heavy metal ions from soil sediments.

The recommended application of these chelators in remediation of mercury-contaminated soils [3] is to be seen in this context. The present data suggest that environmental aspects of chelators and resulting complexes have to be considered in more detail. In particular, aspects of genotoxicity of heavy metal complexes must be introduced into this discussion.

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