

# Tellurite-induced oxidative stress leads to cell death of murine hepatocarcinoma cells

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**Abstract** Data regarding tellurium (Te) toxicity are scarce. Studies on its metabolism, performed mainly in bacteria, underline a major role of reactive oxygen species (ROS). We investigated whether tellurite undergoes redox cycling leading to ROS formation and cancer cell death. The murine hepatocarcinoma Transplantable Liver Tumor (TLT) cells were challenged with tellurite either in the presence or in the absence of different compounds as *N*-acetylcysteine (NAC), 3-methyladenine, BAPTA-AM, and catalase. NAC inhibition of tellurite-mediated toxicity suggested a major role of oxidative stress. Tellurite also decreased both glutathione (GSH) and ATP content

by 57 and 80%, respectively. In the presence of NAC however, the levels of such markers were almost fully restored. Tellurite-mediated ROS generation was assessed both by using the fluorescent, oxidation-sensitive probe dichlorodihydrofluorescein diacetate (DCHF-DA) and electron spin resonance (ESR) spectroscopy to detect hydroxyl radical formation. Cell death occurs by a caspase-independent mechanism, as shown by the lack of caspase-3 activity and no cleavage of poly(ADP-ribose)polymerase (PARP). The presence of  $\gamma$ -H2AX suggests tellurite-induced DNA strand breaking, NAC being unable to counteract it. Although the calcium chelator BAPTA-AM did show no effect, the rapid phosphorylation of eIF2 $\alpha$  suggests that, in addition to oxidative stress, an endoplasmic reticulum (ER) stress may be involved in the mechanisms leading to cell death by tellurite.

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## Introduction

Tellurium (Te) exists in three main forms in the environment: elemental (Te<sup>0</sup>), inorganic [telluride (Te<sup>2-</sup>), tellurite (TeO<sub>3</sub><sup>2-</sup>), tellurate (TeO<sub>4</sub><sup>2-</sup>)] and organic [dimethyl telluride (CH<sub>3</sub>TeCH<sub>3</sub>), dimethyl ditelluride (CH<sub>3</sub>TeTeCH<sub>3</sub>)] (Chau and Wong 1986; Feldmann et al. 1994; Hirner et al. 1998; Chasteen and Bentley 2003). The excretion of DMTe and

trimethyltelluronium (TMTe) in the breath and urine, respectively, has been reported after ingestion of Te at substantial doses (Ogra et al. 2007; Taylor 1996). DMTe and DMDTe are the only relatively volatile organotellurium compounds stable enough to be detected in gas emissions into the atmosphere. The use of Te compounds has found many applications in the manufacture of ceramics, glass, semiconductors, and metals. In addition, it has been used in alloys with germanium (Ge), antimony (Sb), and/or bismuth (Bi) in phase-change optical magnetic disks such as digital versatile disk-random access memory (DVD-RAM) and DVD-recordable disks (DVD-RW) (Yamada et al. 2002).

From a biological point of view, Te is known to be a non-essential but harmful metalloid (Wagner et al. 1995) and its toxic effects and metabolism are poorly understood. The antioxidant properties of some organotellurium compounds have led to further investigate their ability to affect cancer cells (Sailer et al. 1999; Engman et al. 2000; Attebery and Sailer 2002). Indeed, since thioredoxin reductase is overexpressed in many cancer cells (McNaughton et al. 2004; Rigobello et al. 2009), the antioxidant activity of organotellurium compounds may be utilized to inhibit thioredoxin reductase and eventually to stop cancer cell growth (McNaughton et al. 2004). The rationale is that by replacing selenium in selenocysteine or interrupting its synthesis Te leads to disruption of thioredoxin synthesis and cancer cell death by apoptosis (McNaughton et al. 2004; Sailer et al. 2004).

On the other hand, Te may undergo redox cycling leading to the formation of reactive oxygen species (ROS) thus triggering oxidative damage to nucleic acids, proteins and lipids (Chen et al. 2001; Noda et al. 2002; Sailer et al. 2004). Therefore, the aim of this study was to test the hypothesis whether tellurite, the most toxic and soluble oxyanion among Te compounds, undergoes redox cycling thus leading to the generation of an oxidative stress that would induce cell death in eukaryotic cells. To this end, Transplantable Liver Tumor (TLT) cells—a murine hepatocarcinoma cell line—were cultured in the absence or in the presence of tellurite and biochemical and morphological determinations were carried out in order to determine the extent and type of cell death. TLT cells have been previously used to assess both in vivo and in vitro anticancer cytotoxic effects (Duerksen and Connor 1978; Taper et al. 1987; Verrax et al. 2004;

Crokart et al. 2005; Benites et al. 2008). Experimental tests included lactate dehydrogenase (LDH) leakage (cell survival), ATP levels (functional ability of cells), and caspase-3 (DEVDase) activity and cleavage of poly(ADPribose)polymerase (PARP) to assess the nature of the cell demise. The oxidation-sensitive fluorescent probe DCHF-DA and ESR spectroscopy of DEPMPO-OH adducts were used to determine ROS and oxygen-derived free radical formation in tellurite-exposed cells, respectively. To gather further information about the mechanisms leading to cell death by tellurite, additional markers were measured and included Nrf2 activation (oxidative and/or electrophile stress), GSH content (oxidative stress), eIF2 $\alpha$  phosphorylation status (ER stress), H2AX phosphorylation (DNA damage) as well as the use of different compounds as *N*-acetylcysteine (NAC, redox modulator), 3-methyladenine (3-MA, autophagy inhibitor), BAPTA-AM (calcium chelator), and catalase (CAT, antioxidant enzyme).

## Materials and methods

### Chemicals

Potassium tellurite (K<sub>2</sub>TeO<sub>3</sub>), *N*-acetylcysteine (NAC), 3-methyladenine (3-MA), BAPTA-AM, dimethylsulfoxide (DMSO), dichlorodihydrofluorescein diacetate (DCHF-DA), sanguinarine, and catalase were from Sigma; hydrogen peroxide was from Merck. Commercial available antibodies used included Nrf2 (clone H-300, Santa Cruz Biotechnology), PARP (Affinity Bioreagents), eIF2 $\alpha$  and  $\gamma$ -H2AX (Cell Signalling). All other chemicals were ACS reagent grade.

### Cell culture and treatments

Murine hepatocarcinoma Transplantable Liver Tumor (TLT) cells were maintained in culture at 37°C in DMEM/F12 (Dulbecco's modified Eagle medium, Gibco) supplemented with 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100  $\mu$ g/ml) and gentamicin (50  $\mu$ g/ml). Cultures were maintained at a density of 1–2  $\times$  10<sup>5</sup> cells/ml. The medium was changed at 48–72 h intervals. All cultures were maintained at 37°C in a 95% air/5% CO<sub>2</sub> atmosphere with 100% humidity.

TLT cells were incubated for defined time intervals at 37°C in the absence or in the presence of potassium tellurite (at 0.01–1 mM). In addition, cells (both exposed to tellurite and untreated controls) were incubated in the presence of different metabolic inhibitors that were added by separately. These included 3 mM NAC, 10 mM 3-MA, 800 UI of catalase, and 10 µM BAPTA-AM. At defined time intervals cells were removed by centrifugation at 600×g for 8 min before proceeding with the assays described below.

#### Assessment of cell viability

Cell viability was assessed by measuring LDH activity according to the procedure of Wroblewski and Ladue (1955) both in the culture medium and in the cell pellet obtained after centrifugation. Results are expressed as the ratio of released/total LDH activity.

#### Assessment of ATP and GSH content

ATP content was determined using the Roche ATP Bioluminescence Assay Kit CLS II according to procedures described by suppliers. Results are expressed as nmol of ATP/mg protein. Protein concentrations were determined by the procedure of Lowry et al. using bovine serum albumin (BSA) as standard (Lowry et al. 1951). Reduced glutathione (GSH) content was estimated according to the ortho-phthalaldehyde (oPT) method as reported elsewhere (Cohn and Lyle 1966). Results are expressed as nmol GSH/mg protein.

#### Caspase-3 activity

Caspase-3 activity was monitored by cleavage of the specific peptide substrate, Asp-Glu-Val-Asp-AFC (DEVD-AFC), according to the procedure outlined in the instructions for the “FluorAce apopain assay” kit (Biorad). Results are expressed as mUnits. Sanguinarine, a flavonoid inducing apoptosis by caspase-3 activation (Weerasinghe et al. 2001), was used as positive reference compound.

#### Measurement of ROS formation

Two different experimental approaches were used to check whether tellurite induces or not ROS formation

in TLT cells. First, intracellular ROS generation was assessed by using DCHF-DA as described previously, with modifications (Verrax and Buc Calderon 2009). In brief, cells were treated for 1 h to various tellurite concentrations; after two washes with pre-warmed HBSS (Hank’s Balance Salt Solution, Gibco), cells were labeled with 1 µM DCFH-DA and incubated for additional 30 min at 37°C. After several washes with HBSS, cells were suspended in 1 ml of HBSS and sonicated with two pulses of 15 s on ice. One hundred microliters (in triplicate) of the sonicated cell extract were used to determine the fluorescence intensity in 96-well black plates. Fluorescence was quantified by using a Victor X2 fluorimeter (Perkin-Elmer, Turku, Finland). Results were expressed as fluorescence units/mg protein and normalized relative to control as oxidative stress index. Additionally, fluorescence microscope images of treated cells were taken using an Optika (Ponteranica, Italy) fluorescence microscope; image capture was performed using a Moticam 2300 (Motic, Hong Kong, China).

The second approach was aimed at the detection of free hydroxyl radicals by ESR spectroscopy (Anzai et al. 2003). After preincubating TLT cells ( $1 \times 10^6$  cells/ml) with 50 mM of the spin-trap agent DEPMPO (RadicalVision, Marseille, France) for 30 min at 37°C, cells were incubated with different concentrations of tellurite for 10 min at the same temperature. Signal detection was also performed in cells not exposed to tellurite (negative control) or treated with 20 mM H<sub>2</sub>O<sub>2</sub> (positive control). To assure that tellurite-induced hydroxyl radical formation occurred intracellularly, assays were carried out in the presence of medium supplemented with tellurite but cells were omitted. ESR spectra were acquired at room temperature using an EPR Elexsys E540 System (Bruker, Rheinstatten) equipped with a X-Band EPR Super High Q cavity cylindrical resonator (ER 4122SHQE, 10 mm diameter) operating at ~9.5 GHz. A flat cell for aqueous samples (ER 160 FC-Q, Bruker, Germany) was filled with 400 µl of the solution and positioned in the resonator with its flat side perpendicular to the direction of the field. The maximum microwave power level was 16.42 mW. Other parameters were as follows: Center Field 3472 G (347.2 mT) Sweep Width 200 G (20 mT), modulation frequency 100 kHz, Modulation Amplitude 1 G (0.1 mT), Time Constant 40.96 ms, Conversion Time 10.24 ms, Resolution 1024 points, 10 scans.

## Western blot assays

At the indicated times, cells were washed twice with ice-cold Phosphate Buffered Saline (PBS-Gibco) and lysed with a Radio Immunoprecipitation Assay (RIPA) buffer (Pierce) supplemented with a cocktail of protease (Sigma) and phosphatase (Calbiochem) inhibitors. Samples were kept on ice for 20 min and centrifuged at  $13,000\times g$  for 20 min at  $4^{\circ}\text{C}$ . Supernatants were collected and stored at  $-80^{\circ}\text{C}$ . Equal amounts of proteins were subjected to SDS-PAGE (6–15% separating gel) followed by electroblot to nitrocellulose membranes. Membranes were blocked during 1 h in Tris Buffered Saline (TBS) solution (pH 7.4) containing 5% powdered milk protein and then incubated overnight at  $4^{\circ}\text{C}$  with the appropriate antibody. After washing, membranes were exposed 60 min at room temperature to a secondary antibody (Chemicon International) linked to HRP (Horseradish peroxidase). Protein bands were detected by chemiluminescence.

## Data analysis

In general, results were expressed as the mean  $\pm$  standard error of the means (SEM). Differences between the experimental groups were analysed using one-way or two-ways ANOVA tests followed, where appropriate, by a Bonferroni post hoc test. These tests were performed using GraphPad Prism software. *P* values less than 0.05 were considered statistically significant.

## Results

### Hepatocarcinoma cell death by tellurite: effect of some inhibitors

Figure 1a illustrates the percentage of cell death in TLT cell populations, as determined by the % of LDH leakage following various times of exposure to different tellurite concentrations. At low concentrations (0.01 mM) tellurite did not affect importantly cellular viability, even after 24 h of incubation. However, at 0.1 mM tellurite cell death occurs in both a time and dose dependent manner as shown by the significant increase ( $P < 0.05$ ) in the number of cells dying after 24 h of treatment.

To collect additional information about potential mechanisms involved in tellurite-induced cell death, some unrelated compounds having antioxidant properties, chelating activity or autophagy inhibitory effects were tested. Neither 3-MA, BAPTA-AM nor catalase were able to abolish cytotoxicity by tellurite (Fig. 1b). In fact, exposure of carcinoma cells to tellurite in the presence of these compounds resulted in nearly the same amount of cell death, about 80% as compared to 10% to that observed in control, untreated cells. Reinforcing a major role of oxidative stress, the presence of NAC strongly decreased the cytotoxic effect of tellurite (Fig. 1b).

### Tellurite induces ROS formation in TLT cells

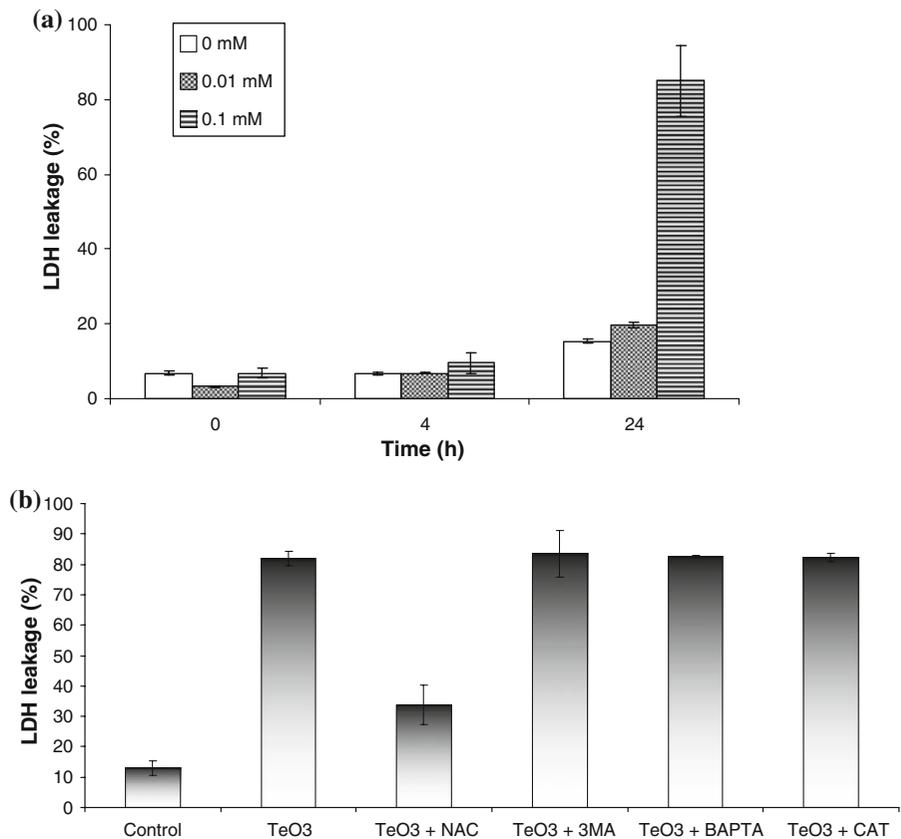
To assess whether tellurite is able to induce oxidative stress, we decided to measure whether ROS formation was occurring inside tellurite-exposed cells. To this end, TLT cells were treated for 1 h with increasing tellurite concentrations (0.01–0.1 mM) and then loaded with DCHF-DA. Figure 2a shows a tellurite concentration-dependent ROS formation, reaching a maximum of 50% at the highest tellurite concentration tested. Similar results were observed in fluorescence images of the treated cells (Fig. 2b).

We also assessed whether tellurite was able to induce the formation of hydroxyl radicals. For this purpose TLT cells were pre-incubated with DEPMPO, a spin-trap able to stabilize free hydroxyl radicals. Figure 2c shows ESR spectra of DMPMPO-OH adducts in cells treated with 1 mM tellurite (middle panel), the signal corresponding to this adduct was confirmed by exposing the cells to 20 mM of  $\text{H}_2\text{O}_2$  (not shown). In order to confirm that hydroxyl radical formation was produced intracellular in cells treated with tellurite, controls with TLT cells in the absence of tellurite (left panel) and without cells were also included (right panel). As with ROS determination assays, the formation of DMPMPO-OH adducts was also dependent on tellurite concentration (not shown).

Does tellurite-induced cell death occur by apoptosis? Is it mediated by oxidative stress?

To check whether apoptosis is activated or not by tellurite, two assays were carried out: caspase-3

**Fig. 1** Effect of tellurite on cell survival. **a** TLT cells were incubated at 37°C in the presence of the indicated tellurite concentrations. At the times shown in the figure, cell suspensions were taken and LDH leakage was measured as described in “Materials and methods”. Values are the mean  $\pm$  SD of at least three different trials. **b** Experimental conditions were similar to those described above. Cells were incubated with 0.1 mM tellurite for 24 h in the presence of the following compounds: *N*-acetylcysteine (3 mM); 3-methyladenine (10 mM); BAPTA-AM (10  $\mu$ M) and catalase (800 UI)



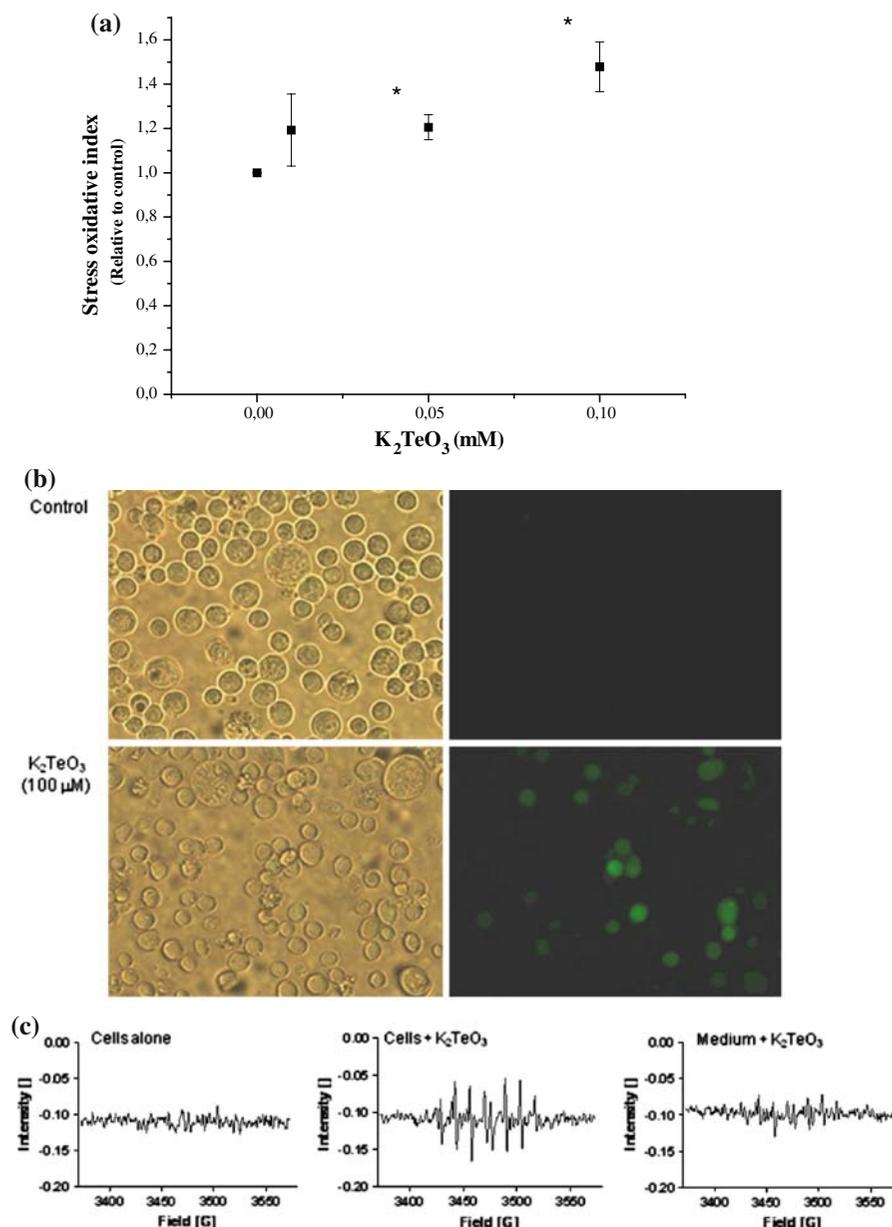
activation (estimated as DEVDase activity) and PARP protein cleavage. No enhancement of caspase-3 activity was observed in tellurite-treated cells (Table 1), while sanguinarine increased the DEVDase activity by ninefold. Incubation of cells in the presence of both tellurite and NAC did not modify caspase-3 activity as compared to control, untreated cells. Furthermore, these effects on caspase-3 activity were confirmed by the integrity of the PARP enzyme (Fig. 3). While sanguinarine fully cleaved PARP, tellurite either alone or in combination with NAC was unable to induce PARP cleavage.

On the other hand, cell exposure to tellurite resulted in a drop of about 50 and 80% of GSH and ATP content, respectively (Table 2). Reinforcing a major role of oxidative stress, NAC strongly decreases tellurite's cytotoxic effects, as shown by 80–100% restoration of GSH and ATP levels regarding control values (Table 2). In addition, Fig. 4 shows the effect of tellurite on some markers reflecting DNA injury (H2AX phosphorylation), endoplasmic reticulum stress (eIF2 $\alpha$

phosphorylation), and cellular adaptive response (nuclear Nrf2 translocation). No changes were observed in the relative amounts of Nrf2, whatever the experimental conditions used (untreated and tellurite-treated cells both in the presence or absence of NAC). The detection of  $\gamma$ -H2AX was observed in tellurite-treated cells but it was not modified by NAC. Figure 4 also shows the effect of tellurite on the phosphorylated status of eIF2 $\alpha$ .

## Discussion

Human fatalities derived from tellurium exposure are rare. Indeed, 3 poisoning-cases have been reported in individuals accidentally injected with sodium tellurite during retrograde pyelography (Keall et al. 1946). Two of those patients died. Their clinical course before death comprised a garlic odor, cyanosis, vomiting, loss of consciousness, and apnoea. Exposure of experimental animals to Te can cause a variety of



**Fig. 2** ROS formation by tellurite. **a** TLT cells were treated with different concentrations of  $K_2TeO_3$  for 1 h, washed, and incubated with DCHF-DA for 30 min. ROS determination was expressed as fluorescence units/mg protein in crude extracts; results are normalized as relative to control (oxidative stress index) and represent the mean  $\pm$  SD ( $n = 3$ ). \*Statistically different ( $P < 0.05$ ) from control. **b** Experimental conditions were similar to those describe above. ROS formation in whole

TLT cells was assessed by fluorescence microscope images (*left panels*, phase contrast; *right panels*, fluorescence). A representative image of three independent assays is shown. **c** Hydroxyl radical spectrum obtained by EPR measurements of TLT cells treated in the absence (*left panel*) or in the presence of 1 mM  $K_2TeO_3$  (*middle panel*). A control in which the medium was supplemented with tellurite in the absence of cells was included (*right panel*)

toxic effects, including reversible hind limb paralysis due to demyelination of the sciatic nerve and spinal roots (Lampert et al. 1970) as well as neurotoxic

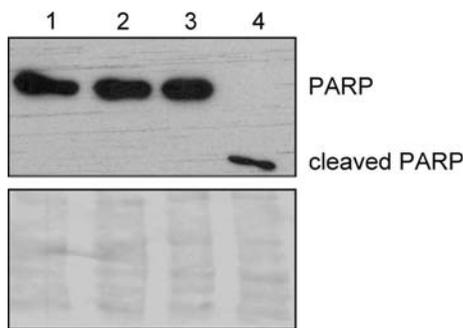
symptoms such as significant impairment of learning and spatial memory (Widy-Tyszkiewicz et al. 2002; Stangherlin et al. 2006).

**Table 1** Caspase-3 activity in the presence of tellurite and sanguinarine

	Activity (mU)
Control	6.3 ± 0.83
Tellurite	5.1 ± 0.21
Tellurite + NAC	5.3 ± 0.65
Sanguinarine	58.5 ± 4.32*

TLT cells were incubated at 37°C for 4 h with 0.1 mM potassium tellurite in the presence or absence of 3 mM NAC and with 10 μM sanguinarine. Aliquots of cell suspensions were withdrawn and the activity of caspase-3 was measured as described in “Materials and methods”. Results are expressed as mU and represent the mean values ± SEM of three different trials

\* *P* < 0.05 as compared to control conditions



**Fig. 3** What type of cell death is induced by tellurite? TLT cells were incubated for 4 h at 37°C without added compounds (lane 1), with 0.1 mM tellurite in the absence (lane 2) or in the presence of 3 mM NAC (lane 3), and with 10 μM sanguinarine (lane 4). Cell suspensions were removed washed, lysed and proteins from supernatants were subjected to SDS-PAGE. Immunoblotting against PARP protein was performed as indicated in “Materials and methods”. Immunodetection was performed using the ECL™ detection kit. The amount of protein loaded in each lane was assessed by staining the blots with Ponceau S (lower panel). A representative blot of three experiments is shown

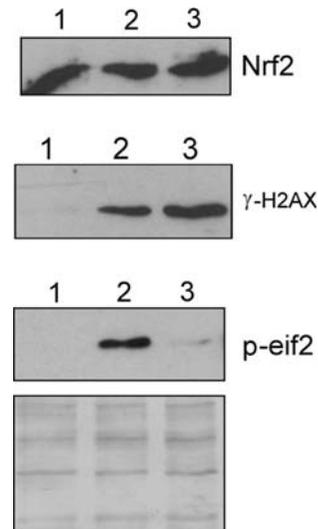
Also, it has been previously shown that HeLa cells incubated for 2 h with 4.5 μM tellurite showed no cytotoxic effects, but by either increasing tellurite concentrations up to 0.27 mM or extending incubation times (24 h), more than 50% of cells died (Ding et al. 2002). Conversely, in ovarian cancer cells incubated for 48 h, tellurite at 5 μM did not induce cell death (Rigobello et al. 2009). In agreement with these reports our results show that tellurite cytotoxicity for TLT cells is dependent on its concentration and exposure time (Fig. 1a). In addition, tellurite

**Table 2** Effects of tellurite on ATP and GSH levels in TLT cells

	ATP (nmol/mg prot)	GSH (nmol/10 <sup>6</sup> cells)
Control	5.9 ± 0.83	0.97 ± 0.12
Tellurite	1.2 ± 0.12*	0.42 ± 0.09*
Tellurite + NAC	4.6 ± 0.06	1.18 ± 0.11

TLT cells were incubated at 37°C for 4 h with 0.1 mM potassium tellurite in the presence or absence of 3 mM NAC. Aliquots of cell suspensions were withdrawn and the amount of both ATP and GSH were measured as described in “Materials and methods”. Results are mean values ± SEM of three different trials

\* *P* < 0.05 as compared to control conditions



**Fig. 4** Effects of tellurite on Nrf2, γ-H2AX and eIF2α. TLT cells were incubated at 37°C for 4 h without any added compound (lane 1), with 0.1 mM tellurite in the absence (lane 2) or in the presence of 3 mM NAC (lane 3), and with 10 μM sanguinarine (lane 4). Cell suspensions were removed washed, lysed and proteins from supernatants were subjected to SDS-PAGE. Immunoblotting was performed as indicated in “Materials and methods”. Immunodetection was performed using the ECL™ detection kit. The amount of protein loaded in each lane was assessed by staining the blots with Ponceau S (lower panel). A representative blot of three experiments is shown

seems to act as a potent cytotoxic agent rapidly affecting some critical metabolic functions such as the maintenance of both ATP and GSH levels (Table 2). The use of some compounds acting on specific targets allowed us to identify more precisely the type of injury caused by tellurite (Fig. 1b). Based

on the lack of effect by 3-methyladenine, a well-known inhibitor of this process (Seglen and Gordon 1982) a major role of macro-autophagy was ruled out. Although the molecular links between impairment of calcium homeostasis and oxidative stress have not been totally elucidated, the lack of effect of BAPTA-AM, a well-known calcium chelator, makes it unlikely a raise of free cytosolic calcium as a mechanism involved in tellurite-mediated cytotoxicity. Furthermore, since catalase does not interfere with tellurite's cytotoxicity, an extracellular production of  $H_2O_2$  is unlikely involved in the mechanism(s) leading to cancer cell death by tellurite. Given that the redox modulator NAC exhibited a strong protective effect, it may be argued that tellurite-redox cycling occurs inside TLT cells, which in turn leads to intracellular ROS formation.

Although organotellurium-induced intracellular ROS accumulation has been reported to be the cause of cell death in HL-60 and MCF7 cancer cells (McNaughton et al. 2004; Sailer et al. 2004), this is the first report showing that tellurite induces the formation of free radicals, particularly hydroxyl radical, in eukaryotic cells (Fig. 2).

While induction of apoptosis in HL-60 cells by organotellurium compounds has been suggested as the type of cell death (Sailer et al. 2004), our results show that neither caspase-3 activity nor PARP cleavage (Table 1 and Fig. 3), two hallmarks of apoptosis, were observed in tellurite-treated TLT cells. These results are in agreement with those recently reported by Rigobello et al. (2009) who demonstrated that tellurite causes a slight decrease in GSH content without inducing apoptosis or cell cycle modifications in ovarian cancer cells exposed to tellurite. Most probably, the chemical nature of the tellurium compounds utilized, namely organotellurium in HL-60 and MCF7 cells and potassium tellurite in TLT and ovarian cancer cells, could account for these apparently opposing results.

On the other hand, Nrf2 enables adaptation to oxidants (Itoh et al. 1997) and electrophiles (Moinova and Mulcahy 1999) by stimulating the transcriptional activation of cytoprotective genes and also the acquisition of cancer resistance to chemotherapy (Lau et al. 2008; Wang et al. 2008). We observed Nrf2 activation both in control and tellurite-exposed cells, where NAC showed no effect (Fig. 4). This is due most probably because hepatocarcinoma TLT

cells, like most cancer cells, have already high basal levels of Nrf2.

If ROS accumulation is one of the mechanisms involved in tellurite-mediated cytotoxicity, we were unable to observe a protective effect against DNA lesions by NAC (Fig. 4). Indeed, ROS (and more probably hydrogen peroxide) could reach the nucleus and cause DNA damage as shown by the strong phosphorylation of histone H2AX, namely  $\gamma$ -H2AX, a marker of DNA strand breaks (Verrax et al. 2004). One hypothetical explanation for this lack of protective effect is that NAC is not reaching the nuclear compartment quantitatively as to stop on time the free radicals-mediated deleterious effects on DNA. Finally, it was further shown that tellurite was able to strongly activate eIF2 $\alpha$  (Fig. 4), a factor tightly regulating protein synthesis and rapidly activated during endoplasmic reticulum (ER) stress. Oxidative stress by tellurite may result in misfolded and/or injured proteins leading to the activation of the endoplasmic reticulum protein kinase PERK. By phosphorylating eIF2, PERK mediates the translational control arm of the unfolded protein response, namely UPR, a complex regulatory process involved in both transcription and translation of a great number of genes (Schroder and Kaufman 2005). Thus, in addition to ROS accumulation, tellurite appears to activate an ER stress.

Although both oxidant damage and ER stress may contribute to tellurite cytotoxicity, the current mechanism by which Te induces cell death is unknown. Based on the chemical similarities between tellurium and selenium, the intracellular mechanism of Te action might be comparable to that proposed for Se-containing compounds (Chasteen and Bentley 2003; Chasteen et al. 2009). Indeed, due to its redox switch activity, tellurite exposure leads to an accumulation of oxygen free radicals within the cytoplasm and nucleoplasm of the cell. The presence of oxygen free radicals in turn leads to an accumulation of DNA single strand breaks, which could activate the machinery associated with apoptotic cell death (Lu et al. 1994). Furthermore, oxygen free radicals can lead to lipid peroxidation (Zhu et al. 1996; Harrison et al. 1997) and to polyamine biosynthesis interruption (Redman et al. 1997), both of which being able to trigger cell death. A key and unique role of oxygen free radicals should, however, be taken with caution since the redox modulator compound NAC did not

show protective effect on tellurite-mediated DNA injury ( $\gamma$ -H2AX, Fig. 4). Another possibility is that Te could be incorporated into amino acids in place of selenium (e.g. selenomethionine and selenocysteine), but no known instances of this occurring in eukaryotic organisms have been reported (Stadtman 1974, 1980). While the experiments described here did not involve measurement of Te incorporation into proteins, we believe that this hypothesis is rather unlikely since a recent report shows that at least in rats, Te metabolism is distinct from that of Se (Ogra et al. 2008). Moreover and contrasting with diphenyl diselenide (PhSe)<sub>2</sub>, diphenyl ditelluride (PhTe)<sub>2</sub> is extremely toxic to rodents and causes marked neurotoxic effects in mice after acute or prolonged exposure (Nogueira et al. 2001; Maciel et al. 2000; Moretto et al. 2005).

Even though the ultimate mechanism of tellurite cytotoxicity is still elusive, data reported here show that tellurite is a potent cytotoxic and most probably a necrosis-like-inducing agent. Likely, this cytotoxicity would occur by combining both oxidant injury and ER stress. Most important, our data point out to the presence of the Te moiety as the key to cellular cytotoxicity. Further use of these compounds in research and industry along with subsequent environmental disposal should take place with caution to prevent potential detrimental effects.

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## References

- Anzai K, Aikawa T, Furukawa Y, Matsushima Y, Urano S, Ozawa T (2003) ESR measurement of rapid penetration of DMPO and DEPMPO spin traps through lipid bilayer membranes. *Arch Biochem Biophys* 415:251–256
- Attebery M, Sailer BL (2002) Detection of apoptosis occurring in promyelocytic (line HL-60) cell populations induced by novel organotellurium compounds. *BIOS* 73:52–60
- Benites J, Rojo L, Valderrama JA, Taper H, Buc Calderon P (2008) Part I: effect of vitamin C on the biological activity of two euryfurylbenzoquinones on TLT, a murine hepatoma cell line. *Eur J Med Chem* 43:1813–1817
- Chasteen TG, Bentley R (2003) Biomethylation of selenium and tellurium: microorganisms and plants. *Chem Rev* 103:1–25
- Chasteen TG, Fuentes DE, Tantaleán JC, Vásquez CC (2009) Tellurite: history, oxidative stress, and molecular mechanisms of resistance. *FEMS Microbiol Rev* 33:820–832
- Chau YK, Wong PTS (1986) Organic group VI elements in the environment. In: Craig PJ (ed) *Organometallic compounds in the environment*. Wiley & Sons, New York, p 274
- Chen F, Vallyathan V, Castranova V, Shi X (2001) Cell apoptosis induced by carcinogenic metals. *Mol Cell Biochem* 222:183–188
- Cohn VH, Lyle JA (1966) Fluorimetric assay for glutathione. *Anal Biochem* 14:434–440
- Crookart N, Radermacher K, Jordan BF, Baudelet C, Cron GO, Grégoire V, Beghein N, Bouzin C, Feron O, Gallez B (2005) Tumour radiosensitization by anti-inflammatory drugs: evidence for a new mechanism involving the oxygen effect. *Cancer Res* 65:7911–7916
- Ding WJ, Hasegawa T, Peng D, Hosaka H, Seko Y (2002) Preliminary investigation on the cytotoxicity of tellurite to cultured HeLa cells. *J Trace Elem Med Biol* 16:99–102
- Duerksen JD, Connor KW (1978) Periodicity and fragment size of DNA from mouse TLT hepatoma chromatin and chromatin fractions using endogenous and exogenous nucleases. *Mol Cell Biochem* 19:93–112
- Engman L, Kanda T, Gallegos A, Williams R, Powis G (2000) Water-soluble organotellurium compounds inhibit thioredoxin reductase and the growth of human cancer cells. *Anti-Cancer Drug Design* 15:323–330
- Feldmann J, Grumping R, Hirner AV (1994) Determination of volatile metal and metalloid compounds in gases from domestic waste deposits with GC/ICP-MS. *J Anal Chem* 350:228–234
- Harrison PR, Lanfear J, Wu L, Fleming J, McGarry L, Blower L (1997) Chemopreventive and growth inhibitory effects of selenium. *Biomed Environ Sci* 10:235–245
- Hirner AV, Krupp E, Schulz F, Koziol M, Hofmeister W (1998) Organometal(loid) species in geochemical exploration: preliminary qualitative results. *J Geochem Explor* 64:133–139
- Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, Nabeshima Y (1997) An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem Biophys Res Commun* 236:313–322
- Keall J, Martin N, Tunbridge R (1946) A report of three cases of accidental poisoning by sodium tellurite. *Br J Ind Hyg* 3:175–176
- Lampert P, Garro F, Pentschew A (1970) Tellurium neuropathy. *Acta Neuropathol* 15:308–317
- Lau A, Villeneuve NF, Sun Z, Wong PK, Zhang DD (2008) Dual roles of Nrf2 in cancer. *Pharmacol Res* 58:262–270
- Lowry O, Rosebrough N, Farr L, Randall R (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 183:265–275
- Lu J, Kaeck M, Jiang C, Wilson AC, Thompson HJ (1994) Selenite induction of DNA strand breaks and apoptosis in

- mouse leukemic L1210 cells. *Biochem Pharmacol* 47: 1531–1535
- Maciel E, Bolzan RC, Braga AL, Rocha JB (2000) Diphenyl diselenide and diphenyl ditelluride differentially affect delta-aminolevulinic acid dehydratase from liver, kidney, and brain of mice. *J Biochem Mol Toxicol* 14:310–319
- McNaughton M, Engman L, Birmingham A, Powis G, Cotgreave IA (2004) Cyclodextrin-derived diorganyl tellurides as glutathione peroxidase mimics and inhibitors of thio-reductase and cancer cell growth. *J Med Chem* 47:233–239
- Moinova HR, Mulcahy RT (1999) Up-regulation of the human gamma-glutamylcysteine synthetase regulatory subunit gene involves binding of Nrf-2 to an electrophile responsive element. *Biochem Biophys Res Commun* 261: 661–668
- Moretto MB, Funchal C, Zeni G, Rocha JB, Pessoa-Pureur R (2005) Organoselenium compounds prevent hyperphosphorylation of cytoskeletal proteins induced by the neurotoxic agent diphenyl ditelluride in cerebral cortex of young rats. *Toxicology* 210:213–222
- Noda Y, Suzuki T, Kohara A, Hasegawa A, Yotsuyanagi T, Hayashi M, Sofuni T, Yamanaka K, Okada S (2002) In vivo genotoxicity evaluation of dimethylarsinic acid in Muta<sup>TM</sup> Mouse. *Mutat Res* 513:205–212
- Nogueira C, Rotta L, Perry M, Souza D, Da Rocha J (2001) Diphenyl diselenide and diphenyl ditelluride affect the rat glutamatergic system in vitro and in vivo. *Brain Res* 906:157–163
- Ogra Y, Kobayashi R, Ishiwata K, Suzuki KT (2007) Identification of urinary tellurium metabolite in rats administered sodium tellurite. *J Anal At Spectrom* 22:153–157
- Ogra Y, Kobayashi R, Ishiwata K, Suzuki KT (2008) Comparison of distribution and metabolism between tellurium and selenium in rats. *J Inorg Biochem* 102:1507–1513
- Redman C, Xu JJ, Peng Y, Scott JA, Payne C, Clark LC, Nelson MA (1997) Involvement of polyamines in selenomethionine induced apoptosis and mitotic alterations in human tumour cells. *Carcinogenesis* 18:1195–1202
- Rigobello MP, Gandin V, Folda A, Rundlöf AK, Fernandes AP, Bindoli A, Marzano C, Björnstedt M (2009) Treatment of human cancer cells with selenite or tellurite in combination with auranofin enhances cell death due to redox shift. *Free Radic Biol Med* 47:710–721
- Sailer BL, Prow T, Dickerson S, Watson J, Liles N, Patel SJ, Van Fleet-Stalder V, Chasteen TG (1999) Bacterial cytotoxicity and induction of apoptosis in promyelocytic (line HL-60) cells by novel organotellurium compounds. *Environ Toxicol Chem* 18:2926–2933
- Sailer BL, Liles N, Dickerson S, Sumners S, Chasteen TG (2004) Organotellurium compound toxicity in a promyelocytic cell line compared to non-tellurium-containing organic analogue. *Toxicol In Vitro* 18:475–482
- Schroder M, Kaufman RJ (2005) The mammalian unfolded protein response. *Annu Rev Biochem* 74:739–789
- Seglen PO, Gordon PB (1982) 3-Methyladenine: specific inhibitor of autophagic/lysosomal protein degradation in isolated rat hepatocytes. *Proc Natl Acad Sci USA* 79:1889–1892
- Stadtman TC (1974) Selenium biochemistry. *Science* 183:915–922
- Stadtman TC (1980) Selenium-dependent enzymes. *Annu Rev Biochem* 49:93–110
- Stangherlin EC, Favero AM, Zeni G, Rocha JB, Nogueira CW (2006) Exposure of mothers to diphenyl ditelluride during the suckling period changes behavioural tendencies in their offspring. *Brain Res Bull* 69:311–317
- Taper HS, de Gerlache J, Lans M, Roberfroid M (1987) Non-toxic potentiation of cancer chemotherapy by combined C and K3 vitamin pre-treatment. *Int J Cancer* 40:575–579
- Taylor A (1996) Biochemistry of tellurium. *Biol Trace Elem Res* 55:231–239
- Verrax J, Buc Calderon P (2009) Pharmacologic concentrations of ascorbate are achieved by parenteral administration and exhibit antitumoral effects. *Free Radic Biol Med* 47:32–40
- Verrax J, Cadrobbi J, Marques C, Taper H, Habraken Y, Piette J, Buc Calderon P (2004) Ascorbate potentiates the cytotoxicity of menadione leading to an oxidative stress that kills cancer cells by a non-apoptotic caspase-3 independent form of cell death. *Apoptosis* 9:223–233
- Wagner M, Toews AD, Morell P (1995) Tellurite specifically affects squalene epoxide: investigations examining the mechanism of tellurium-induced neuropathy. *J Neurochem* 64:2169–2176
- Wang XJ, Sun Z, Villeneuve NF, Zhang S, Zhao F, Li Y, Chen W, Yi X, Zheng W, Wondrak GT, Wong PK, Zhang DD (2008) Nrf2 enhances resistance of cancer cells to chemotherapeutic drugs, the dark side of Nrf2. *Carcinogenesis* 29:1235–1243
- Weerasinghe P, Hallock S, Tang SC, Liepins A (2001) Role of Bcl-2 family proteins and caspase-3 in sanguinarine-induced bimodal cell death. *Cell Biol Toxicol* 17:371–381
- Widy-Tyszkiewicz E, Piechal A, Gajkowska B, Smialek M (2002) Tellurium-induced cognitive deficits in rats are related to neuropathological changes in the central nervous system. *Toxicol Lett* 131:203–214
- Wroblesky F, Ladue J (1955) Lactic dehydrogenase activity in blood. *Proc Soc Exp Biol Med* 90:210–213
- Yamada N, Kojima R, Uno M, Akiyama T, Kitaura H, Narumi K, Nishiuchi K (2002) Phase-change material for use in rewritable dual-layer optical disk. *Proc SPIE* 4342:55–63
- Zhu Z, Kimura M, Itokawa Y, Aoli T, Takahashi JA, Nakatsu S, Oda Y, Kikuchi H (1996) Apoptosis induced by selenium in human glioma cell lines. *Biol Trace Elem Res* 54:123–134