Anti-cancer drugs interfere with intracellular calcium signaling

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ABSTRACT

Toxicity of metal and metal compounds is frequently highlighted. While specific metals or metal compounds are essential for cellular function, other metals are toxic and/or carcinogenic. Metals can trigger accidental cell death in the form of necrosis, or activate programmed cell death in the form of apoptosis.

The aim of anti-cancer therapy is induction of apoptosis in tumor cells. Therefore, there is an interesting twist in the toxicity of metals and metal compounds (e.g., arsenic trioxide, cisplatin); since they have a higher specificity to induce apoptosis in cancer cells (possibly due to the high turnover in these cells) they are used to cure some forms of cancer.

A body of evidence suggests that second messengers, such as modulations in the intracellular calcium concentration, could be involved in metals induced toxicity as well as in the beneficial effects shown by anti-cancer drugs.

Here we review the influence on calcium homeostasis induced by some metallic compounds: cisplatin, arsenic trioxide and trimethyltin chloride.

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1. Intracellular calcium ([Ca²⁺]i) as an intracellular messenger

Intracellular calcium ([Ca²⁺]i) is a second messenger and signal transducer in both excitable and non-excitable cells. It is involved in physio- and pathological processes. Under normal conditions, the intracellular calcium concentration is maintained at 10–100 nM, but sustained Ca²⁺ release from calcium stores, Ca²⁺ influx through receptor- or voltage-dependent calcium channels or blockage of re-uptake can perturb [Ca²⁺]i homeostasis. A modulation of [Ca²⁺]i is an important factor in the activation of accidental or programmed cell death.

A variety of metals and metal compounds are able to modify [Ca²⁺]i signaling and therefore might induce necrosis or apoptosis. While necrosis can occur following a calcium overload, apoptosis is

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1.1 Intracellular calcium ([Ca²⁺]i) as an intracellular messenger

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A variety of metals and metal compounds are able to modify [Ca²⁺]i signaling and therefore might induce necrosis or apoptosis. While necrosis can occur following a calcium overload, apoptosis is
a highly regulated process found in multicellular organisms. Apoptosis is involved in normal physiological regulation as well as in diseases such as inflammation, malignancy, autoimmunity and neurodegeneration. Regulation of the apoptotic process is critical for cancer development and for treatment. While defective apoptosis could lead to uncontrolled cell growth and tumor formation, the induction of apoptosis by anti-cancer drugs might be crucial for cancer treatment. Generally, disorders of apoptosis may play a vital role in hepatotoxicity, renal toxicity, neurotoxicity, autoimmunity and carcinogenesis (Desoize, 2002a; Florea et al., 2005a,b,c; Florea and Büßelberg, 2009).

\([\text{Ca}^{2+}]\) dynamics are modulated by active and passive transport mechanisms. The classical calcium signaling theory affirms that the increase in \([\text{Ca}^{2+}]\), can be due to (1) calcium entry from the extracellular space, through calcium selective channels in the plasma membrane; or (2) \(\text{Ca}^{2+}\) release from intracellular calcium stores. The decrease of \([\text{Ca}^{2+}]\), is established by (1) plasma membrane pumps or exchangers at the outer cellular membrane; or (2) through re-entry to the calcium stores (Fig. 3B), represented mainly by mitochondria and endoplasmic reticulum, via \(\text{Ca}^{2+}\)-transport systems (most likely active transport mechanisms driven by co-transport or by the use of ATP). Intracellular \(\text{Ca}^{2+}\) could also be bound by calcium-buffering proteins that can further modulate \([\text{Ca}^{2+}]\). One of the most important events resulting from \(\text{Ca}^{2+}\) signaling is the activation of biological pathways that are modulated by the binding of calcium to calcium-sensor proteins (Mattson et al., 2000; Berridge et al., 2003; Orrenius et al., 2003).

The calcium stores (ER) play important roles in \([\text{Ca}^{2+}]\), homeostasis and signaling like: (a) regulating crucial processes (e.g., motility, secretion, gene expression); (b) signaling cascades that drive proliferation, differentiation, and various metabolic reactions; and (c) cell death in physiological settings or during injury or disease (Mattson et al., 2000; Berridge et al., 2003; Orrenius et al., 2003; Desoize, 2002a; Florea and Büßelberg, 2006, 2009; Florea, 2007).

In addition an important mechanism in which toxic metallic entities might interfere with calcium homeostasis of cells is the direct binding to sulphydryl groups of proteins (including enzymes, channels, and pumps) resulting in conformational changes and impaired function of channels and pumps. Furthermore, arsenic could indirectly affect the functioning of enzymes, proteins, channels and pumps by reducing the antioxidant capacity (GSH levels) of the cell, ensuing increased protein oxidation and altered protein function (for review see Qian et al., 2003; Florea et al., 2005a,b,c; Florea and Büßelberg, 2006).

Overall, intracellular \(\text{Ca}^{2+}\) signaling is essential for the function of cells and tissues while metals can impair \([\text{Ca}^{2+}]\)-dependent processes and therefore resulting in pathological conditions and toxicity (for review see Florea and Büßelberg, 2006).

### 2. Relevance of metals and metal compounds for human health

Metals and metal compounds are commonly present in the environment. Humans actively circulate and distribute metals through agricultural and industrial applications such as the manufacture of pesticides, batteries, alloys, metal parts, textile dyes, steel, etc. (reviewed in Florea and Büßelberg, 2005, 2006, 2009; Florea, 2007). All organisms including humans are affected by metal exposure as metals can enter the body through food, water, air, or absorption through the skin.

Some metals are essential for biochemical processes, for example, zinc is an important cofactor for several enzymatic reactions while haemoglobin contains iron. In small quantities, certain metals known as trace elements (e.g., iron, copper, manganese, selenium, zinc) are nutritionally essential and are found in fruits, vegetables and food supplements such as multi-vitamin products (reviewed in Florea and Büßelberg, 2006; Florea, 2008).

Nevertheless, metal concentrations exceeding physiological levels could be toxic since they are able to generate free radicals that result in lipid peroxidation as well as the depletion of the sulphhydryl groups. Metal toxicity can present in humans as allergies, neurological disorders, or damage to vital organs while long-term exposure could result in cancer and/or death (for review see Florea, 2005; Florea, 2008).

Since metals and metal compounds modify \([\text{Ca}^{2+}]\), homeostasis they alter signal transduction pathways and induce DNA damage and inhibit DNA repair. The inhibition of DNA repair could occur directly by free radical formation or substitution of zinc in Zn-finger domains, as well as indirectly by lowering the level of reduced glutathione (Desoize, 2002a; Florea et al., 2005a,b,c). Activation of nuclear factor-kappa B (NF-κB) as a transcription factor in response to oxidative stress is another way metal compounds can induce toxicity since NF-κB-controls genes involved in crucial processes such as inflammation, carcinogenesis and anti-apoptotic reactions. In turn, inhibition of NF-κB causes impairment of cell cycle progression (Desoize, 2002a).

### 3. The use of metal based compounds as anti-cancer drugs

Metals and metal compounds have been used in medicine for thousands of years, even eliciting serious side effects. For example, while platinum complexes are known to be toxic they are also some of the best anti-cancer drugs. Similarly, arsenic trioxide can be used as an anti-cancer drug but can also induce tumors.

Here we highlight how platinum compounds and arsenic are used in anti-cancer therapy, followed by an overview of different mechanisms by which metal compounds change the \([\text{Ca}^{2+}]\), and therefore induce apoptosis. This process might be beneficial for cancer treatment when specific for tumor cells.

#### 3.1. Platinum compounds

Platin complexes are clinically used to treat various types of cancers, including sarcomas, carcinomas, lymphomas and germ cell tumors. The most commonly used platinum compounds are cisplatin (CDDP, Platinol), carboplatin and oxaliplatin (Desoize, 2002b; Desoize and Madoulet, 2002). These, along with other platinum compounds, induce damage to tumors via induction of apoptosis which is mediated by the activation of various signal transduction pathways. The effect of platinum compounds is related to the number of platinum adducts formed with the DNA that consequently inhibits DNA synthesis and repair via modification of the three-dimensional structure of the DNA (Desoize, 2008b; Desoize and Madoulet, 2002).

Furthermore, this can result in cell cycle arrest at the G1,S, or G2–M phases and therefore the induction of apoptosis occurs—an important caveat considering cell cycle turnover is much greater in cancer cells (Sorenson and Eastman, 1988; Johnson et al., 1997; Trimmer and Essigmann, 1999; Guggenheim et al., 2009; Henkels and Turchi, 1997, 1999; Desoize, 2004).

As stated earlier, different platin compounds are used for different types of cancers because each varies in its efficiency for a specific tumor. Presently, it has been demonstrated in chemotherapies that cisplatin is superior to carboplatin in treatment of testis, bladder, head and neck, small cell lung cancer (SCLC) and in several paediatric malignancies whereas in other cancer types, cisplatin has tended to replace carboplatin. Much of the selectivity in chemotherapies is also based on the compound’s relative toxicity. In the case of carboplatin, it is selected for clinical use because of its lower non-hematological toxicity compared to cisplatin. Oxaliplatin in clinical use is higher both in its efficacy and lower in toxicity as compared to cisplatin. Another platinum based
chemotherapy, nedaplatin, was developed because it produced better results in preclinical trials. Nedaplatin is generally used for myelo suppression with licensed indications for head and neck, testicular, lung (NSCLC and SCLC), oesophageal, ovarian, and cervical cancer (Desoizze, 2002b).

The currently accepted paradigm for the mechanism of action for cisplatin is that the drug induces its cytotoxic properties through binding to nuclear DNA and subsequently interferences with normal transcription, and/or DNA replication mechanisms. Cisplatin induces apoptosis by increased caspase-8 activity, upregulation of p53 expression, cleavage of Bid to its truncated form, activation and mitochondrial translocation of Bax, induction of mitochondrial permeability, release of cytochrome c into the cytosol, activation of caspase-9, and entry into the execution phase of apoptosis (Henkels and Turchi, 1999). Cisplatin is also involved in proteolytic degradation of procaspase-3 and caspase-3 activation, increases in cytoplasmic cytochrome c, decreases of Bcl-2 and increases in Bcl-xl all leading to programmed cell death (Henkels and Turchi, 1999). Unfortunately, death receptor mechanisms as well as mitochondrial pathways in cisplatin-induced apoptosis are also involved in cisplatin-induced toxicity (Devarajan et al., 2002; Park et al., 2002; Jiang et al., 2004). Moreover, there is evidence suggesting that the cytotoxic effects induced by the binding of cisplatin to non-DNA targets may contribute to its biochemical mechanism of action (Fuertesa et al., 2003; Cepeda et al., 2007).

Ever since the discovery of the anti-cancer activity of cisplatin, major efforts have been devoted to elucidate the biochemical mechanisms involved—this all in the hope to rationally design novel platinum based drugs with superior pharmacological profiles. The factors that influence cisplatin therapy include: drug uptake, DNA damage in signal transduction, and cell death. Understanding the biochemical mechanisms triggered in tumor cells in response to cisplatin may not only lead to the design of more efficient platinum antitumor drugs but also provide new therapeutic strategies based on the biochemical modulation of cisplatin activity (Cepeda et al., 2007; Gonzalez et al., 2001).

3.2. Arsenic

Arsenic compounds have been used as medicinal agents for many centuries for the treatment of diseases such as psoriasis, syphilis, rheumatism, plague, hysteria, hypertension, trypanosomiasis, bleeding gastric ulcers, heartburn, and chronic rheumatism and cancer. The discovery in the 1980s that arsenic trioxide induces complete remission in a high percentage of patients with acute promyelocytic leukemia has increased the interest in this metalloid for the treatment of human disease such as hematological malignancies and solid tumors. Currently, As$_2$O$_3$ is considered as the treatment of choice for patients with relapsed acute promyelocytic leukemia (APL) (Dilda and Hogg, 2007; Roboz, 2008; Aronson, 1994; Antman, 2001; Efferth et al., 2007).

Arsenic has applications in industry and agriculture and such activity is a major source of the environmental contamination associated with arsenic compounds. The correlation between arsenic exposure, cytotoxicity and genotoxicity, mutagenicity, and tumor promotion has been established (for review see Florea et al., 2005a,b,c). Arsenic exposure has also been recognized in the perturbation of physiologic processes such as calcium homeostasis, generation of reactive oxygen species, DNA damage, and apoptosis induction. Different possible modes of action of arsenic-induced carcinogenesis have been proposed: chromosomal damage, oxidative stress, modification of gene expression, modulation of DNA repair or DNA methylation and interactions with growth factors or cell proliferation. This is due in part to the fact that As$_2$O$_3$ can also mobilize cell defense mechanisms by activating pro-survival molecules such as MEK-ERK, Bcl-xl, and Bcl-2. Arsenic exposure may also result in tumor promotion/progression, gene amplification, suppression of p53, as well as global DNA hypomethylation or malignant transformation (Kitchin, 2001; Zhao et al., 1997; Florea et al., 2005a,b,c). Trivalent forms of arsenic have been found to induce apoptosis in several cellular systems with the involvement of membrane-bound cell death receptors, caspase activation, calcium store release, and changes of the intracellular glutathione level. Besides arsenic's toxic and carcinogenic effects, arsenic is used to treat malignancies demonstrating a paradoxical effect: arsenic is more toxic in tumor cells as compared with normal cells (Florea et al., 2005a,b,c; Florea and Büsselberg, 2006, 2009).

Mammals are able to metabolise arsenic. It has been shown that following cellular uptake, inorganic arsenic undergoes biotransformation to mono- and dimethylated metabolites. This process is a toxification pathway because trivalent organoarsenic compounds are more potent in inducing cytotoxicity and DNA damage than the inorganic precursors in “in vitro” cell models—a fact that is correlated with the uptake capability of cells (Florea, 2005; Florea et al., 2005a,b,c). The cellular uptake of arsenic compounds was a matter of debate for some time, but it has been shown that in vitro test systems, cellular uptake is very low; a fact that strongly influenced the induction of cytotoxicity and DNA damage. This has been demonstrated through a forced uptake of arsenic compounds by electroporation, which significantly increased the toxic effects (Florea, 2005; Florea et al., 2005a,b,c). Thus, direct exposure to trivalent organic derivatives might not be the highest concern in regard to arsenic toxicity because the half-life of these compounds is relatively short. But, toxic arsenic metabolites are produced within cells upon the biotransformation of inorganic arsenic and therefore could directly and strongly affect physiological processes such as signaling pathways (Florea, 2005; Florea et al., 2005a,b,c).

It has been suggested that the primary mechanism by which arsenicals manifest cell injury/death and cancer is through the inhibition of mitochondrial respiration supported by NAD-linked substrates that use the lipoic acid cofactor for the pyruvate dehydrogenase complex (NRC, 1999). Consequently, this process can induce oxidative stress and generate the reactive oxygen species (ROS) which may cause DNA mutations and could be strongly related to the development of cancer. Arsenic toxicity can elicit numerous intracellular processes or changes within the cell that may lead to apoptosis. These include but are not limited to intracellular Ca$^{2+}$ disturbances, PKC activation, mitochondrial depolarization and depletion of intracellular GSH, upregulation of caspase-3, down regulation of Bcl-2, Bax/Bak-dependent release, activation of c-jun – N-terminal kinase (JNK) pathway, deficiency of p53, activation of AP-1 and NF-$kappa$B, DNA strand breaks, increased levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) (reviewed in Rana, 2008; Liu et al., 2005; Li et al., 2006; Yu et al., 2008). Interestingly, as-induced cell death occurs through a p53-independent pathway in p53 deficient cells, while in normal tissue, apoptosis induction occurs through a p53-dependent pathway (Yu et al., 2008) which could represent evidence for the differential effects of arsenic. A recent study using tumor and non-tumor cell models has shown that there are no major differences in As$_2$O$_3$ influence on [Ca$^{2+}$]i between the two types, however, the effect is stronger in tumor cells as compared to non-tumor cells. Therefore, it could be that the fine regulation of calcium stores may be involved in specific effects shown by arsenic or that calcium signals trigger different signaling pathways in the two cell types (Florea et al., 2007; Florea and Büsselberg, 2008; Florea, 2007).

Tallman (2008) has also discussed the dual mechanisms of action of As$_2$O$_3$ in treatment of acute promyelocytic leukemia (APL): (1) activation of the apoptotic caspase cascade via the intrinsic or mitochondrial pathway and (2) the induction of differentiation via PML/RAR alpha. Treatment with As$_2$O$_3$ leads to the release of cytochrome c from the mitochondria, which induces
the activation of caspase-3 and programmed cell death while As$_2$O$_3$ also specifically degrades the PML/RARα fusion transcript that allows the release of RARα, transcription of RARα target genes, and differentiation (Tallman, 2008).

### 3.3. Trimethyltin chloride (TMT)

While TMT is not an anti-cancer drug it shares some common mechanisms with the two compounds described before, therefore it might be interesting to differentiate why TMT effects to our knowledge are solely toxic. Tin compounds are ubiquitously distributed in the environment especially enriched in aquatic ecosystems being involved in the food chain which can affect human health. Tin compounds have high environmental persistence, mobility, and lipophilicity resulting in bioaccumulation in food webs. Biotransformation of organotin compounds has been detected in the environment where alkyl group removal has been attributed to the action of UV light, chemical cleavage, and biological degradation by bacteria. In mammalian organs such as the brain, liver, and kidneys, organotins are progressively dealkylated to inorganic tin (reviewed in Flores, 2005).

Some organotin compounds were found to be cytotoxic, genotoxic, and mutagenic as well as neurotoxic. Because of their lipophilicity, organotins are regarded as membrane active. There is evidence that the site of action of organotins may be both at the plasma membrane as well as in the intracellular space. It is not known, however, whether cell surface adsorption or accumulation within the cell or both is the reason for toxicity (for review see Flores, 2005; White et al., 1999). Several organotin compounds exert an antineoplastic effect (Syng-ai et al., 2002) and some compounds are used in nuclear medicine where they are employed as a reducing agent for technecium-99m (99mTc) and, therefore, applied intravenously (Florea and Büsselferg, 2006).

TMT is a toxic organotin compound that produces injury to the central nervous systems of mammals. Recently, high-dose TMT (2.8 mg/kg) has been shown to produce neurodegeneration and subsequent neurogenesis specifically in the hippocampal dentate gyrus of mice. The involvement of stannin, nuclear factor-kappa B (NF-κB), presenilin-1, apolipoprotein E, and pituitary adenyl cyclase-activating polypeptide (PACAP) in TMT toxicity have been demonstrated and suggest a relationship between genetic mutations and neuronal susceptibility to degeneration (Shintani et al., 2007).

TMT intoxication is considered as a suitable experimental model to study the molecular basis of selective hippocampal neurodegeneration that occurs in several neurodegenerative diseases (Placentini et al., 2008). TMT causes lesions in defined regions of the hippocampus and neocortex (Patanow et al., 1997). TMT determines specific apoptotic destruction of pyramidal neurons in the CA3 region of the hippocampus and in other limbic structures. Expression of the protein stannin is required for the development of TMT-induced lesions but stannin alone is insufficient in induction of apoptotic pathways in neuronal populations. But TMT induce oxidative stress, neuronal damage and myelin edema, respectively (Philbert et al., 2000). TMT causes oxidative stress in cultured cells which is believed to be the cause of a large part of the destructive actions disturbing calcium homeostasis, mitochondrial activity and inhibition of ATP synthesis altering the structure and function of membranes (for review Flores, 2005; Flores and Büsselferg, 2006).

### 4. Cisplatin, arsenic trioxide and TMT trigger an increase of [Ca$^{2+}$]

It has been found that the elevation of [Ca$^{2+}$] is a common mechanism of the three metal compounds. Nevertheless there are some major differences, which are important in understanding the beneficial as well as toxic effects of these substances.

#### 4.1. Cisplatin elevates [Ca$^{2+}$] by an increased Ca$^{2+}$ uptake from the extracellular space in some tumor cells

Numerous metal cations, particularly divalent (e.g., Pb$^{2+}$, Zn$^{2+}$, Sn$^{2+}$) are known for reducing the Ca$^{2+}$-current through voltage gated calcium channels (Büselferg et al., 1994a,b; Büsselferg, 1995; Tomaszewski and Büsselferg, 2008), which result in lowering the level of [Ca$^{2+}$]. Recently, we could demonstrate that the anti-cancer drug cisplatin (cis-diamin-di-chloro-platin=CDDP) also reduces the voltage activated calcium currents especially of small dorsal root ganglion neurons in a concentration-dependent manner (1–100 µM) with an IC$\text{50}$ value of about 24 µM, while large neurones are less sensitive (Tomaszewski and Büsselferg, 2007; Fig. 1A).

While voltage gated calcium channel currents are reduced by cisplatin, [Ca$^{2+}$] increases when cisplatin is applied (Kawai et al., 2006; Splettstoesser et al., 2007). While both studies describe [Ca$^{2+}$]; elevation that they are contradictory with respect to the mechanism proposed. While Kawai et al. (2006) demonstrated in a renal non-tumor cell model that cisplatin in a concentration range between 250 and 750 µM induced the [Ca$^{2+}$], elevation by a Ca$^{2+}$ release from the intracellular calcium stores, Splettstoesser and colleagues postulated a Ca$^{2+}$ entry from the extracellular space (Fig. 1B) in low concentrations (0.001–10 µM) in a specific tumor cell line. Cisplatin in this concentration range elevates [Ca$^{2+}$], in HeLa-S3 (human cervix adenocarcinoma) but not in U2-OS (human osteosarcoma cells).

The CDDP induced rise of [Ca$^{2+}$] is concentration dependent and mediated by IP$_3$ receptors (Fig. 1C). Extracellular Ca$^{2+}$ is essential and the Ca$^{2+}$ concentration in the stores increases when the drug is applied. In addition, the [Ca$^{2+}$], increase is associated with apoptotic death and was dependent on the activation of calpain but not caspase-8. Since the correlation between the increase of [Ca$^{2+}$], and the induction of apoptotic cell death has been determined, we conclude that an IP$_3$ receptor-dependent calcium influx triggered by CDDP results in the activation of calpain which can possibly end with programmed cell death. This is at least one possible signal pathway required for cisplatin-induced apoptosis. Results also suggest that the efficiency of cisplatin treatment could be enhanced if [Ca$^{2+}$], could be increased pharmacologically, possibly by an independent activation of IP$_3$ receptors (Splettstoesser et al., 2007). Interestingly, Tachikawa et al., 1998 has given some indication that resistance of tumor cells could be directly related with lower CDDP amount found in the cells and diminished effects on calcium homeostasis induced by CDDP, a fact that supports our hypothesis (Tachikawa et al., 1998).

Overall, the effect of clinically relevant concentrations of cisplatin on calcium homeostasis of different tumor cells is important in optimizing cancer treatment regimes.

#### 4.2. Arsenic trioxide and trimethyltin chloride (TMT) share a common mechanism: both trigger a Ca$^{2+}$ release from the stores

Low environmental and clinically relevant concentrations of As$_2$O$_3$ (0.1 nM to 1 µM) interfere with [Ca$^{2+}$], homeostasis of neuroblastoma (SY-5Y) and human embryonic kidney (HEK 293) cells. As$_2$O$_3$ induces a concentration- and time-dependent elevation of [Ca$^{2+}$], that is not reversible if As$_2$O$_3$ is removed (Fig. 2). Three forms of [Ca$^{2+}$], elevations were found: (1) steady-state increases; (2) transient [Ca$^{2+}$], elevations; and (3) [Ca$^{2+}$], spikes. The [Ca$^{2+}$], rise has been shown to be dependent on the internal calcium stores (Fig. 2) and on the calcium related receptors inositol triphosphate (IP$_3$) and ryanodine (Ry) receptors. This suggests that
the calcium stores and calcium receptors are a primary target of arsenic induced deregulation while the disturbance of $[\text{Ca}^{2+}]_{\text{i}}$ homeostasis is related to reduced cell viability, DNA damage and apoptosis in both cell types. Thus As$_2$O$_3$-induced cell death could be triggered or mediated by $[\text{Ca}^{2+}]_{\text{i}}$ signals and suggests that Ca$^{2+}$ is a main regulator of As$_2$O$_3$-induced toxicity however, other mechanisms could also be involved (Florea et al., 2007; Florea and Büsselberg, 2008, 2009).

Unfortunately, there is little known about arsenic uptake in vivo. But, to introduce toxic effects, arsenic compounds do not necessarily need to be taken up and biotransformed since they could trigger death mechanisms by interacting with the cell membrane or simply by small amounts of arsenic passing through the membrane. Such instances could be sufficient to modify other physiological processes such as calcium homeostasis (Florea et al., 2005a,b,c; Florea and Büsselberg, 2006, 2009). An important question that arose from this study is whether As$_2$O$_3$ needs to enter the cytosol to accomplish the rise of $[\text{Ca}^{2+}]_{\text{i}}$. Previously it was documented that arsenite, an inorganic trivalent arsenic form, has a very low uptake in vitro (below 5% of the substrate (Florea, 2005)). Since As$_2$O$_3$ is structurally very similar to arsenite, this provides an indication that As$_2$O$_3$ does not need to enter the cell to complete its effect but could directly interact with receptors at the cell membrane, thereby increasing $[\text{Ca}^{2+}]_{\text{i}}$, and activating signaling pathways. This conclusion is underlined by the ability of sub-nanomolar concentrations of As$_2$O$_3$ to induce a $[\text{Ca}^{2+}]_{\text{i}}$ rise in neuroblastoma and HEK cells, which were concentration- and time-dependent. Thus, experiments applying liposomes containing As$_2$O$_3$ in order to increase the intracellular amount of As$_2$O$_3$ did not result in a higher cytotoxicity which was probably due to a fast export from the cells. Consequently, it could be speculated that As$_2$O$_3$ interaction with various cell models occurs in two different ways. Either: (1) As$_2$O$_3$ binds to receptors at the extracellular membrane triggering intracellular pathways that result in an elevated intracellular Ca$^{2+}$ level; and/or (2) a relatively small amount of As$_2$O$_3$ entering the cytosol is sufficient to release Ca$^{2+}$ from the stores.

In other work regarding the interaction of As$_2$O$_3$ with tumors cells, the involvement of mitochondria on the rise of $[\text{Ca}^{2+}]_{\text{i}}$ was demonstrated (Ma et al., 2006; Shen et al., 2002). On the other hand, little or no investigations have been conducted to analyze the involvement of the ER in As$_2$O$_3$-induced $[\text{Ca}^{2+}]_{\text{i}}$ rise and cell death. Only the study of Zheng et al. (2005) using microarray technology have underlined the importance of calcium signaling and the ER in As$_2$O$_3$-induced effects in tumor cells. The control of Ca$^{2+}$ dynamics are complex and are regulated not only by ion channels and pumps at the plasma membrane but the ER could play a major role in the regulation of rapid and long-term changes in $[\text{Ca}^{2+}]_{\text{i}}$, (e.g., Ca$^{2+}$ is released from the ER in response to signals that activate receptors coupled to IP$_3$ production) (Mattson et al., 2000; Berridge et al., 2003).

Similarly, TMT induces a concentration-dependent sustained elevation of $[\text{Ca}^{2+}]_{\text{i}}$, as well as transient intracellular Ca$^{2+}$ rises in vitro while the number of reacting cells is directly related to the concentration of TMT. In contrast to As$_2$O$_3$, the $[\text{Ca}^{2+}]_{\text{i}}$ increase was partly reversible. The fast $[\text{Ca}^{2+}]_{\text{i}}$ transients (spikes) were observed even with the lowest TMT concentration tested (0.25 μM). The calcium rise also originates from internal stores and did not significantly depend on the presence of Ca$^{2+}$ in the extracellular...
space (Fig. 2). Staining of calcium stores with rhod-2 has shown a TMT-induced $[\text{Ca}^{2+}]_i$-decrease in the stores followed by an increase of the calcium concentration in the nuclei which could be related to cytotoxicity or genotoxicity induced by TMT (Florea, 2005; Florea et al., 2005a,b). Thus, the cyto-/genotoxicity of alkylated tin compounds has become a matter of debate due to low cellular uptake in in vitro systems while forced uptake by electroporation induced significant DNA damage at non-cytotoxic concentrations (Florea, 2005).

In addition, it has been shown that rat hippocampal neurons expressing the $\text{Ca}^{2+}$ binding protein calretinin (CR) are spared by the neurotoxic action of TMT hypothetically due to their ability to buffer intracellular $\text{Ca}^{2+}$ overload. In cultured rat hippocampal neurons, TMT produces time- and concentration-dependent intracellular $\text{Ca}^{2+}$ increases that are primarily due to $\text{Ca}^{2+}$ release from intracellular stores although $\text{Ca}^{2+}$ entry through $\text{Ca}^{2+}$ entry channels also contributed to $[\text{Ca}^{2+}]_i$ increases in the early phase of TMT action (Placentini et al., 2008).

5. While cisplatin and arsenic trioxide both increase $[\text{Ca}^{2+}]_i$, their consecutive application further increases $[\text{Ca}^{2+}]_i$ and consequently elevates apoptosis

5.1. Issues in combination therapies

Combination therapy has gained much interest in the push towards effective cancer treatments. Fortunately, these therapy strategies also have the ability to diminish the effect of pro-survival molecules and the manner in which this is done varies for both arsenic and platinum based chemotherapies. In these cases, arsenic compounds can activate cellular defense mechanisms while platinum complexes can develop cross-resistances which render the drugs ineffective.

Arsenic compounds have been shown to be effective in activating pro-apoptotic pathways. Unfortunately, the same compounds can activate pro-survival molecules that allow certain cancers to essentially adapt to any further chemotherapy. However, there are certain drugs that target specific pro-survival molecules and thus are capable of enhancing pro-apoptotic pathways which may lead to an improvement of $\text{As}_2\text{O}_3$ efficacy against hematological malignancies. As previously mentioned, arsenic can activate MEK-ERK, Bcl-xL, and Bcl-2 pro-survival mechanisms. However, it has been shown that by combining $\text{As}_2\text{O}_3$ with specific MEK inhibitors that block MEK-ERK phosphorylation, the induction of Bad de-phosphorylation, and activation of the p53AIP1 apoptotic pathway interrupt the pro-survival mechanisms of ATO and kill leukemic cancer cells by apoptotic synergism (Bonati et al., 2006; Ohnishi, 2007; Wang and Chen, 2008).

Regarding platin complexes, preliminary success in tumor regression is often followed by recurrence and resistance to any further chemotherapeutic treatment. With the various platinum based cancer drugs comes the ability for the tumor to develop a resistance and subsequent cross-resistance with additional platin chemotherapies. Carboplatin, although low in toxicity, exhibits a high cross-resistance with its parent compound. Nedaplatin with its outstanding preclinical trials still maintains cross-resistance to other platin compounds including cisplatin. Of the four platin compounds discussed previously, oxaliplatin is the only complex which does not exhibit cross-resistance. This compound in particular has developed some interesting results in a number of cancers especially in colorectal cancer. Furthermore, oxaliplatin efficacy is remarkable against cancers resistant to other platinum derivatives (Boulikas and Vougioouka, 2003; Desoize and Madoulet, 2002; Akaza et al., 2001; Hartmann and Lipp, 2003).

The attraction of cisplatin, beside its efficacy, is that its toxicity is different from that of other anti-cancer drugs, a characteristic favoring its use in combination therapy. The major limitation in the clinical applications of cisplatin, as with other platin chemotherapies, has been the development of cisplatin resistance by tumors. Factors affecting the occurrence of cisplatin resistance include: increased drug efflux, decreased drug influx, increased cellular glutathione levels, increased DNA repair, drug tolerance and the execution of apoptosis. Mechanisms explaining cisplatin resistance include the reduction in cisplatin accumulation inside cancer cells because of barriers across the cell membrane, faster repair of cisplatin adducts the modulation of apoptotic pathways in various cells, the upregulation in transcription factors, the loss of p53 and other protein function and a higher concentration of glutathione and metallothioneins in some types of tumors. A number of experimental strategies to overcome cisplatin resistance are at the preclinical or clinical level such as through the introduction of the...
Cisplatin as well as arsenic trioxide affect $\text{Ca}^{2+}$, by different mechanisms and trigger apoptosis (Desoize, 2004; Florea and Büsselberg, 2006). Therefore, combination of these two drugs could improve anti-cancer effects; while As$_2$O$_3$ releases calcium from the internal stores (Fig. 2), cisplatin triggers calcium uptake from the extracellular space (Fig. 1). As described above, a single application of As$_2$O$_3$ or CDDP increased transient and sustained $\text{Ca}^{2+}$-elevations, while the sustained increase showed clear additive effects when both drugs were applied. The magnitude of the $\text{Ca}^{2+}$ increase depends on the order of application; the most pronounced effect occurred when the cells were preincubated with CDDP followed by a co-application with As$_2$O$_3$ (Fig. 3); while the application of cisplatin opened a calcium channel at the cellular membrane (Fig. 3A), which is most likely IP$_3$ receptor related, the increase of $\text{Ca}^{2+}$, activated calcium extrusion mechanisms which actively pump the calcium back to the extracellular side simultaneously move $\text{Ca}^{2+}$ to the calcium stores (Fig. 3B). Therefore, the calcium concentration in the stores is also elevated after about 1 h of incubation (Spletstoesser et al., 2007). While the presence of cisplatin keeps the calcium conductance at the outer cellular membrane open, the additional application of As$_2$O$_3$ also activates a calcium release from the (filled) stores (Fig. 3C).

Sustained $\text{Ca}^{2+}$ elevations result in an increased cytotoxicity and apoptosis. Therefore, co-treatment with CDDP and As$_2$O$_3$ may be a more effective anti-cancer therapy then either agent alone (Fig. 4; Günst et al., 2009).

6. Conclusions

Metal compounds clearly have toxic effects. Several of the mechanisms have been associated with disturbances of the intracellular calcium concentration. While this alters $\text{Ca}^{2+}$-dependent pathways and could explain many of the toxic effects of metal compound, an elevation of $\text{Ca}^{2+}$ could also be a crucial signal to induce apoptosis.

Cancer cells have a relatively high rate of mitosis, this might give them a higher vulnerability to disturbances of the intracellular calcium concentration and therefore the use of drugs (here metal compounds) which will elevate $[\text{Ca}^{2+}]_i$ could result in a higher apoptotic rate of these cells. When two different mechanisms ($\text{Ca}^{2+}$-influx and $\text{Ca}^{2+}$ release from the stores) could be utilized to increase $[\text{Ca}^{2+}]_i$, even to a higher degree, this might result in a higher apoptotic rate and therefore the elimination of the cancer cells. Thus, it is not clear yet whether these changes are causal changes or secondary changes therefore more work is needed to identify the exact role of $[\text{Ca}^{2+}]_i$ and the possibility of utilizing this possibility for cancer treatment.

Ongoing experiments and clinical trials regard not only the use of newly designed drugs, but also investigate the use of specific combination strategies that could maximize the expected effects in treating malignancies. Despite the immense efforts in cancer research, there are still many unanswered questions that need to be solved urgently in order to improve antitumor treatments.

Conflict of interest statement

No conflict of interest.

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