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Neutral and positively charged thiols synergize the effect of the immunomodulator AS101 as a growth inhibitor of Jurkat cells, by increasing its uptake

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ABSTRACT

The immunomodulator amonium trichloro[1,2-ethanediolato-O,O'] tellurate (AS101), a nontoxic tellurium(IV) compound, exhibited antitumoral activity in several preclinical and clinical studies. In this study, we investigated the synergism between thiols and AS101 in its antitumoral activity on Jurkat cells. AS101 induced a G_2/M arrest in the cell cycle after 24 h. Addition of the thiols 2-mercaptoethanol or cysteamine led to an induction of apoptosis. Other thiols, including glutathione (GSH) and cysteine, did not potentiate the effect of AS101. We propose that this is due to the alpha-carboxylate group present in the compounds formed between AS101 and these thiols. Programmed cell death was associated with the loss of mitochondrial transmembrane potential and activation of caspase-3 and -9. Elevation of intracellular reactive oxygen species (ROS) production was also demonstrated; the antioxidant catalase significantly reduced the apoptosis, suggesting that ROS play a key role in the apoptosis induced by AS101 and the thiols. Finally, we quantified the intracellular concentration of tellurium, using electron microscopy and energy-dispersive spectroscopy (EDS) analysis. The addition of cysteamine to AS101 significantly increased the concentration of tellurium within the cells. The results indicate that neutral or positively charged thiols but not negatively charged ones, increase the antitumoral effect of AS101 by increasing its uptake into the cells.

1. Introduction

Tellurium is a relatively rare element, belonging to the chemical family of oxygen, sulfur, selenium, and polonium (the chalcogens). Significant amounts of tellurium (up to 600 mg) are present in the human body. These levels are higher than all but three of the recognized trace elements (viz., iron, zinc and rubidium) [1], though the physiological functions of

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Abbreviations: AS101, amonium trichloro[1,2-ethanediolato-O,O'] tellurate; PBS, phosphate-buffered saline; BCH, 2-aminobicycloheptane-2-carboxylic acid; GSH, glutathione; 2-ME, 2-mercaptoethanol; EDS, energy dispersive X-ray spectroscopy; SEM, scanning electron microscope; TEM, transmission electron microscope; PE, phycoerythrin; MMP, mitochondrial transmembrane potential; NAC, *N*-acetyl-L-cysteine; CYSM, L-cysteine methyl ester; PI, propidium iodide; ROS, reactive oxygen species

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tellurium remain obscure [2]. The sources of the tellurium present within human tissues are also unclear; accumulation of tellurium in plants, in a manner analogous to that of selenium, has not been demonstrated [3].

The nontoxic immunomodulator amonium trichloro[1,2-ethanediolato-O,O'] tellurate (AS101), first developed by us, is a low molecular weight synthetic tellurium compound. AS101 possesses immunomodulating properties that have shown beneficial effects in several pre-clinical and clinical studies. In a variety of tumor models, AS101 was shown to have a direct anti-tumor effect [4,5] and to improve the survival of Madison lung carcinoma-bearing mice and B16 melanoma tumor-bearing mice when given in combination with chemotherapy. Clinical trials in non-small lung cancer patients treated with AS101 in combination with chemotherapy have shown a significant reduction in the severity of neutropenia and thrombocytopenia that accompanies treatment [6,7].

Tellurium commonly occurs in forms corresponding to oxidation states +6, +4 or −2. Te(IV) compounds interact readily with thiols to form $Te(thiol)_4$ compounds. These undergo redox disproportionation to disulfide and $Te(thiol)_2$ products. The latter is unstable and can further react to a second disulfide and elemental tellurium [8]. Much of this chemistry is common to selenium as well [9].

AS101, as a tellurium(IV) compound, can also interact with thiols to form a Te–S bond. We have shown that many of its biological activities depend on its ability to interact with a cysteine residue in the active site of several proteins, and thus to modify their activity [8,10,11]. The present study was designed to delineate the ability of AS101 to interact with several thiols and induce apoptosis in malignant cells. We further characterize the observed apoptotic process and describe the efficiency of various thiols in enhancing the apoptotic effect of AS101.

2. Materials and methods

2.1. Cell culture

Jurkat cells were grown in RPMI-1640 medium with 10% fetal calf serum supplemented with 100 units/ml penicillin and 100 μg/ml streptomycin (Biological Industries, Kibbutz Beit Haemek, Israel). Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

2.2. Reagents

AS101 was synthesized as previously described [4], dissolved in PBS (pH 7.4) and maintained at 4 °C. Glutathione, 2-mercaptoethanol, cysteine, cysteamine, L-cysteine methyl ester, N-acetyl-L-cysteine, catalase, 2′,7′-dichlorofluorescein diacetate (DCFH-DA) and JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazol-carbocyanine iodide) were all obtained from Sigma–Aldrich.

2.3. Cell cycle and apoptosis analysis

Subconfluent cells were treated with AS101 for 24 h. Cells were harvested, washed with PBS, fixed and resuspended in 70% ethanol at −20 °C overnight. Next, the cells were washed once with PBS and resuspended in Nicoletti buffer [12]. DNA content was analyzed by a FACSCalibur flow cytometer using CellQuest pro software (BD Biosciences). The apoptotic fraction was assessed as the percentage of cells present in the sub-G_1 population.

2.4. Apoptosis determination with annexin/PI

Cells were dual-labeled with annexin V and propidium iodide (Annexin V-FITC Apoptosis Detection kit I, Pharmingen). Labeled cells were acquired in a FACSCalibur flow cytometer (Becton Dickinson), and analysis of cell populations was performed using the CellQuest package.

2.5. Measurement of intracellular ROS

To measure the amount of intracellular ROS generated by the cells, 2′,7′-dichlorofluorescein diacetate (DCFH-DA) was used. This compound is a nonfluorescent, cell-diffusible dye. Intracellular esterases cleave the acetyl groups from the molecule to produce nonfluorescent DCFH. This is trapped inside the cell and in the presence of ROS, DCFH is subsequently modified to fluorescent DCFH, which can be detected by flow cytometry. After the cells were incubated with AS101 and cysteamine, they were preloaded with 10 μM DCFH-DA at 37 °C for 30 min and then washed twice with PBS. Each sample was analyzed using a FACSCalibur. The results show the change in the mean fluorescent intensity (MFI) compared to the untreated controls.

2.6. Analysis of mitochondrial membrane potential

Mitochondrial damage was assessed by JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazol-carbocyanine iodide) staining. This dye, which emits green fluorescence (537 nm) in its monomeric form in solution, can assume a dimeric configuration emitting red fluorescence (597 nm) in a reaction driven by the mitochondrial transmembrane potential [13]. Thus, red fluorescence of JC-1 indicates integral mitochondria, whereas green fluorescence shows monomeric JC-1 that remained unprocessed due to collapse of the mitochondrial membrane potential ($\Delta\psi_m$). Cells were adjusted to a density of 0.2×10^6 ml^{-1}, washed with PBS, resuspended in 1 ml medium, stained with 1 μg/ml JC-1 for 30 min at 37 °C and 5% CO_2 in the dark, then washed twice with PBS and resuspended in 0.5 ml PBS. Analysis was performed by a FACSCalibur flow cytometer and mitochondrial function was assessed based on the ratio of red and green fluorescence [14]. The results show the change in the ratio compared to the untreated controls.

2.7. Caspase-3, -9 assay

Cells were collected to test tubes and centrifuged at 1200 rpm. They were then washed once with cold PBS, resuspended in 500 μl of PBS and fixed in cold 70% ethanol. After 24 h, the cells were washed with PBS and resuspended in blocking solution (2% BSA, 0.5% Tween-20 in PBS)

containing 10 μl phycoerythrin (PE)-conjugated anti-active caspase-3 antibody (BD Biosciences, Palo Alto, CA), and samples were incubated for 30 min at RT. Samples were washed twice, resuspended in 300 μl PBS and analyzed by flow cytometry. The proportion of cells staining for PE above the level of a nonspecific PE-conjugated control antibody was quantified. The percentage of positive cells reacting with each antibody was determined on a FACSCalibur flow cytometer. The presence of active caspase-9 was determined according to the protocol in the Carboxyfluorescein FLICA kit purchased from Immunochemistry Technologies, LLC. This assay is based on the FLICA™ reagent (Fluorescent Labeled Inhibitors of Caspases) and is comprised of 3 segments: a green fluorescent label (FAM = carboxyfluorescein); a peptide inhibitor sequence targeted by active caspase-9 (LEHD); and a fluoromethylketone group (FMK) which acts as an alkylating group and forms a covalent bond with the active enzyme.

2.8. EDS

In electron microscopy, an electron beam is scanned across (SEM) or through (TEM) a sample's surface to obtain an image of the structure under investigation. Interaction of the primary beam with atoms in the sample causes shell transitions which result in the emission of an X-ray. Since the emitted X-ray has an energy characteristic of the parent element, detection and measurement of the energy permits elemental analysis (energy dispersive X-ray spectroscopy or EDS). EDS can provide rapid qualitative, or with adequate standards, quantitative analysis of elemental composition. Cells (6×10^5 cells/ml) were cultured and AS101 and cysteamine were added for 2–4 h; the cells

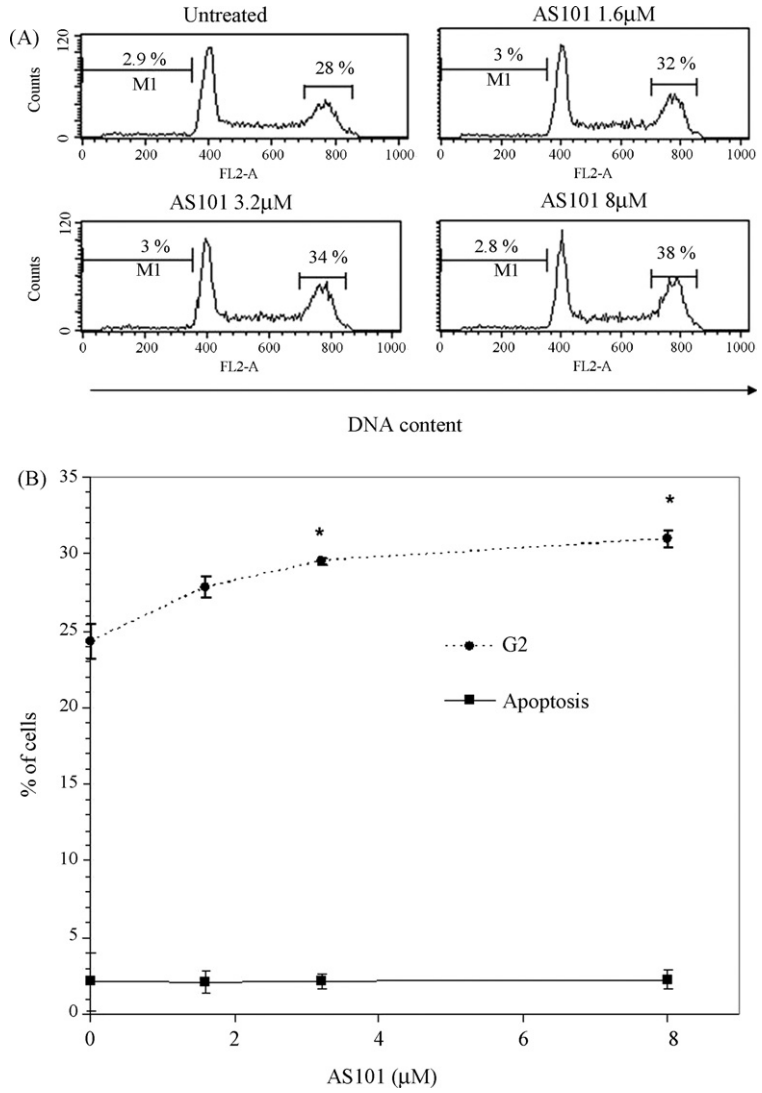

Fig. 1 – Treatment of Jurkat cells with AS101 results in increased accumulation of cells in the G_2/M phase. Cells (5×10^5 ml^{-1}) were treated with increasing amounts (1.6–8 μM) of AS101 for 24 h and cell cycle distribution was assessed. (A) The percentage of cells in each phase of the cell cycle was estimated by flow cytometry analysis. Results show one representative experiment out of three performed. (B) Data represent mean ± S.E. from three independent experiments. * $p < 0.05$ increase vs. untreated cells.

were centrifuged and fixed in 70% ethanol. The fixed cells were centrifuged, and the pellet was resuspended in ethanol and dripped onto a carbon grid for analysis. High-resolution TEM energy dispersive X-ray spectra were obtained with a JEOL 2011 TEM equipped with EDS (Oxford Instruments). INCA energy software was used for the collection and storage of the spectrum and the percentage of tellurium was calculated from the total spectrum of elements measured in the cells. For scanning electron microscopy, spectra were collected with a JEOL JSM-7000F with a Themo Noran System Six energy dispersive X-ray spectrometer.

2.9. Statistical analysis

Data are presented as mean ± S.E. Comparisons between treatment groups were made using either paired *t* tests or ANOVA and Dunnett T3 post hoc tests. Significance was established at a value of $p < 0.05$.

3. Results

3.1. AS101 alters the cell cycle of Jurkat cells

AS101 exhibits anti-tumor properties in several cancer models [4,5]. In this experiment we tested the effect of AS101 on the cell cycle of Jurkat cells. Jurkat cells were treated with or without 1.6, 3.2 and 8 μM AS101 for 24 h. As the concentration of AS101 increased, the number of cells within the G_2/M populations was increased in a dose dependent manner, displaying significance at 3.2 and 8 μM (Fig. 1).

3.2. AS101 synergizes with 2-mercaptoethanol or cysteamine, but not with GSH and cysteine, to induce apoptosis in malignant cells

We have shown that many of AS101's biological activities depend on its ability to interact with a cysteine residue in the active site of several proteins and thus modify their activity

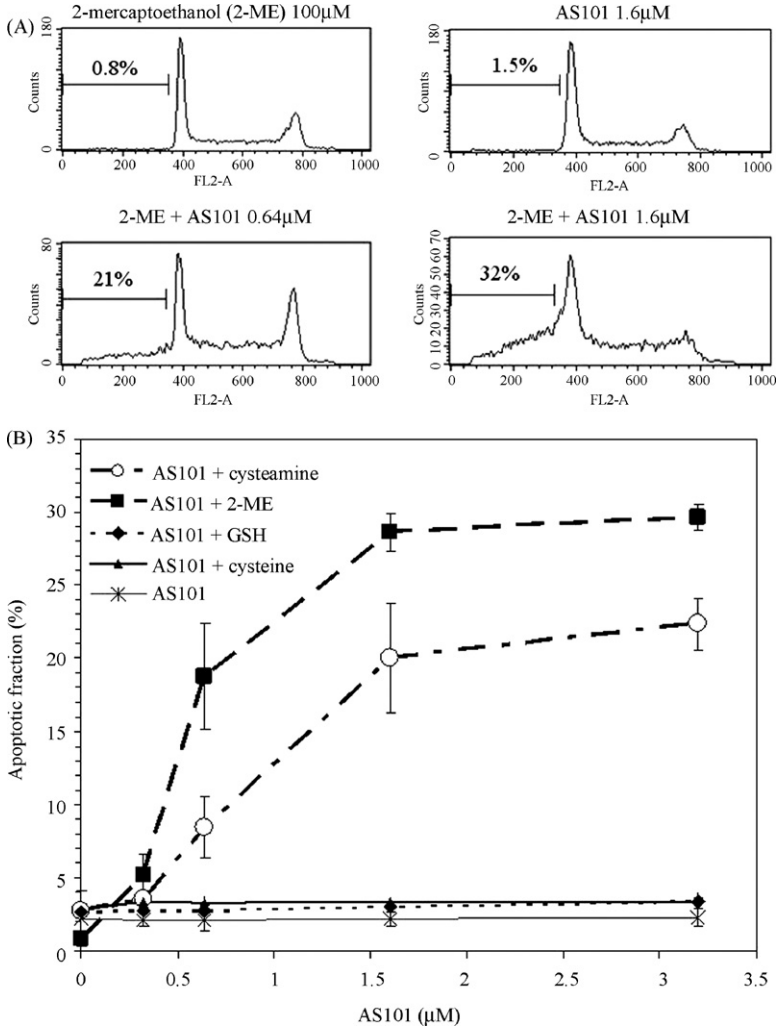

Fig. 2 – AS101 synergizes with 2-mercaptoethanol or cysteamine but not with GSH or cysteine to induce apoptosis in malignant cells. Each of the thiols at 100 μM was added to AS101 (0.32, 0.64, 1.6 and 3.2 μM) for 24 h. (A) The apoptotic fraction (Sub-G1) of 2-ME, AS101 and AS101 plus 2-ME was estimated by flow cytometry analysis. Results show one representative experiment out of three performed. (B) The percentage of apoptotic cells is indicated as the proportion of sub-G1 cells. Data represent mean ± S.E. ($n = 3$).

[8,11]. 2-Mercaptoethanol (2-ME) is an essential part of the primary T-lymphocyte medium and acts as a cystine carrier in L1210 cells which are deficient in the capacity to take it up [15]. We therefore examined whether the addition of 2-ME to the Jurkat cells would modify the effect of AS101. In addition, we tested another thiol, cysteamine, which has a similar effect to 2-ME on T-lymphocytes [16] but is a natural product of cells and was shown to react with selenite to form a selenium compound that is rapidly absorbed by the intestinal epithelium [17]. We also studied the effect of two other physiological thiols, glutathione (GSH) and cysteine. Fig. 2 shows that AS101 together with cysteamine or 2-ME, but not GSH or cysteine, caused dose dependent apoptosis in the Jurkat cells.

In addition to the sub-G_1 population analysis, we investigated the effect of AS101 and cysteamine on annexin V binding, which is an early hallmark of apoptosis. As the dose of AS101 increased from 0.32 to 1.6 μM the number of cells in early apoptosis (annexin V positive, PI negative) was increased from 3% in the control to 17% with 1.6 μM AS101 in a dose dependent manner. In addition, there was also a considerable increase in the late apoptosis (Fig. 3a). AS101 and 2-ME produced similar results (data not shown). To further confirm

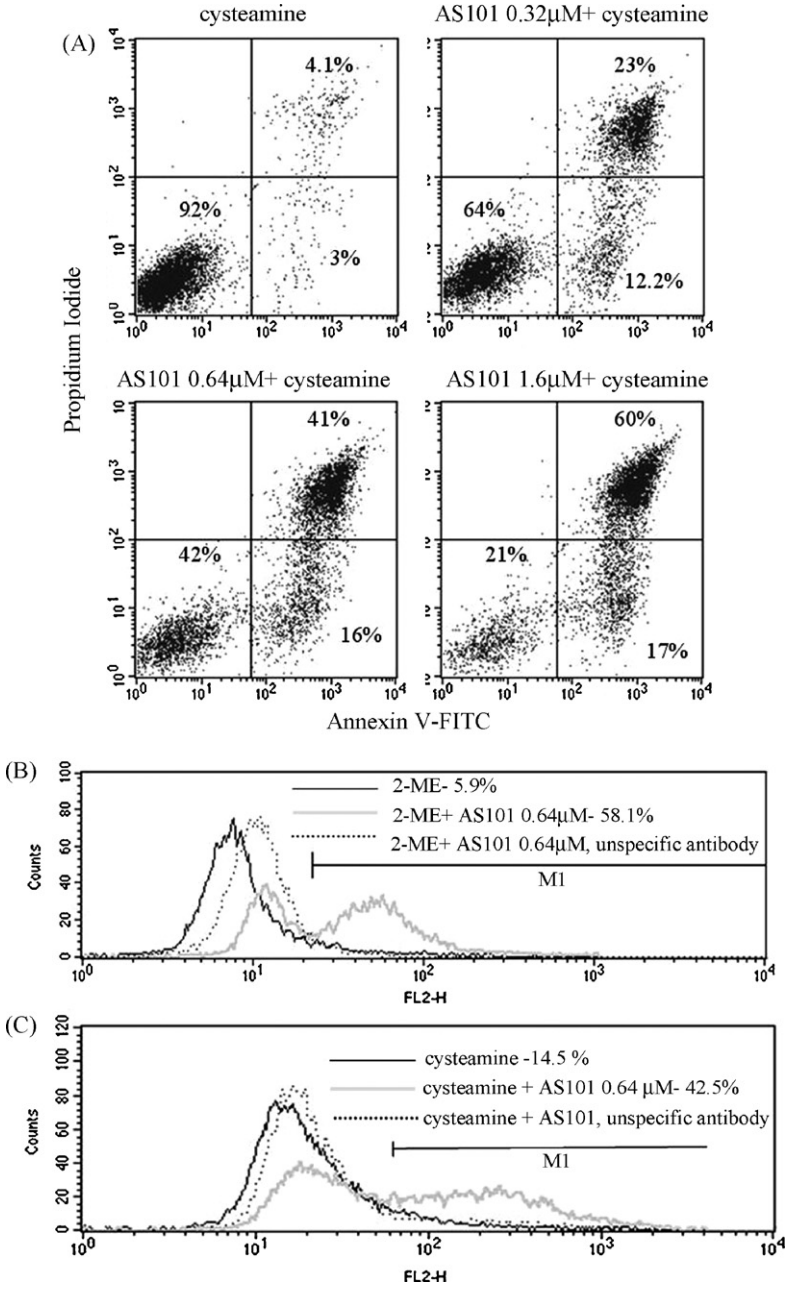

Fig. 3 – AS101 synergizes with 2-ME or cysteamine to induce apoptosis. Jurkat cells were treated with increasing concentrations of AS101 (0.32, 0.64 and 1.6 μM) together with cysteamine (100 μM) for 24 h and cell death was assessed by FACScan analysis of annexin-V–FITC/PI staining (A). Cells treated with AS101 and 100 μM 2-ME (B) or 100 μM cysteamine (C) were analyzed for the processing of caspase-3 as described under Section 2. The results show one representative experiment of three performed.

that AS101 and cysteamine or 2-ME induce apoptosis, we tested caspase activity. These enzymes play a critical role in the execution of apoptosis and are responsible for many of the biochemical and morphological changes associated with it [18,19]; consequently, caspase activity has been widely used to identify cells undergoing apoptosis. Significant increase in the active form caspase-3 was seen in cells treated with AS101 and 2-ME (Fig. 3b), which is consistent with results from the sub-G_1 and annexin assays. Similar results were attained with cysteamine (Fig. 3c). In all the experiments AS101 alone had no effect on the level of apoptosis (data not shown).

3.3. *AS101 together with cysteamine or 2-ME induce apoptosis by increasing ROS and disrupting the mitochondrial membrane potential*

Several studies have suggested that intracellular ROS generation may constitute an apoptotic event and cite ROS production

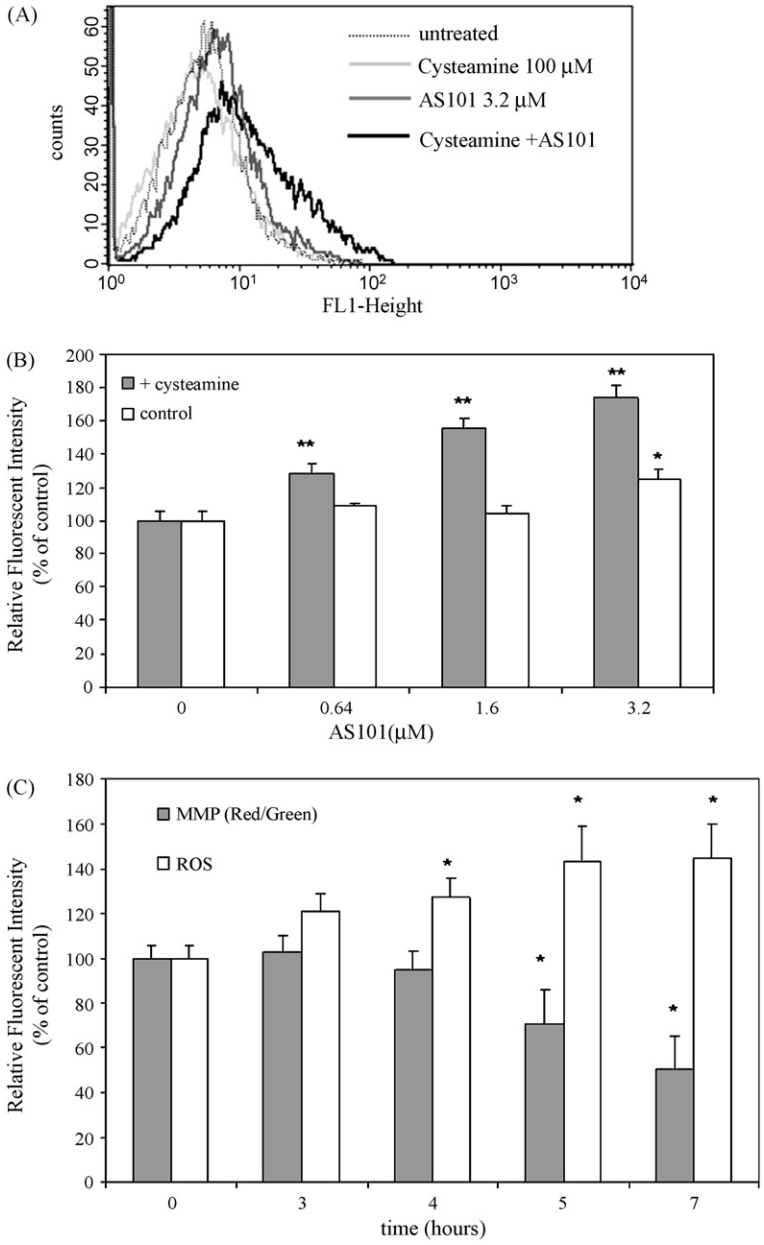

Fig. 4 – AS101 together with cysteamine induce apoptosis by increasing ROS and disrupting the mitochondrial membrane potential. (A) FACScan analysis of Jurkat cells incubated with 3.2 μM AS101 and 100 μM cysteamine for 5 h and then stained with 10 μM DCFH-DA for 30 min. (B) Cells were treated with several concentrations of AS101 with or without 100 μM cysteamine for 5 h and then stained with 10 μM DCFH-DA to determine the level of ROS. (C) Cells were treated with 1.6 μM AS101 and 100 μM cysteamine for different time periods and then stained with either JC-1 to test MMP or 10 μM DCFH-DA to determine the level of ROS. Data are presented as mean ± S.E. from three independent experiments. Comparisons between treatment groups were made using ANOVA and Dunnett T3 post hoc tests. Significance was established at a value of $^{*}p < 0.05$, $^{**}p < 0.01$.

as a critical determinant of toxicity associated with exposure to ionizing radiation and chemotherapeutic drugs [20]. One of the more prominent models for ROS induction suggests that H_2O_2 acts upon mitochondria, causing a disruption of mitochondrial membrane potential (MMP), the release of cytochrome c which then initiates the caspase cascade by first activating caspase-9 and later caspase-3 [21].

We wished to determine whether the apoptotic signal triggered by AS101 and cysteamine has an effect on the level of ROS. Cells were incubated with AS101 and cysteamine for various time periods before staining with DCFH-DA to measure ROS. Results show that when AS101 is added together with cysteamine there is a dose dependent increase in ROS, compared to untreated cells. In addition, AS101 alone (without cysteamine) at 3.2 μM showed a small but significant increase in ROS (Fig. 4a and b). Next, we tested the effect of AS101 and cysteamine on the level of ROS and MMP over time. The results show that both the level of ROS and the number of cells with a depolarized mitochondrial membrane increase over time. However the level of ROS increases somewhat faster and shows a change after 3 h, which is significant after 4 h, in comparison to the reduction in mitochondrial potential, which shows significance only after 5 h (Fig. 4c). To further investigate the role of ROS in the apoptosis induced by AS101 and cysteamine, we used the antioxidant enzyme catalase. Several studies show that catalase can enter cells and carry its antioxidative activity inside [22,23]. The results show that catalase completely blocked the mitochondrial dysfunction induced by AS101 and cysteamine (Fig. 5a). In addition, it also abolished the activation of caspase-9 and substantially reduced the apoptosis as determined by the annexin/PI assay (Fig. 5b and c). We confirmed that catalase normalized the ROS

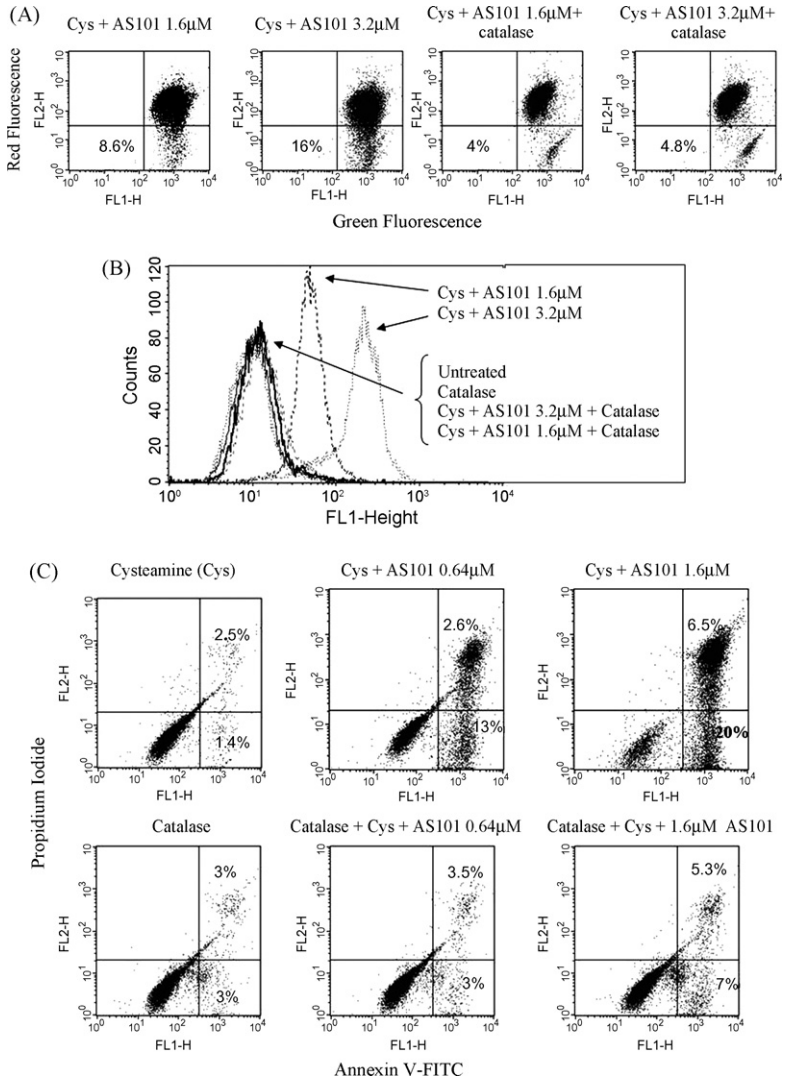

Fig. 5 – The antioxidant enzyme catalase blocked the apoptotic effect of AS101 and cysteamine. (A) Cells were treated with 100 μM cysteamine, alone or together with 1.6 or 3.2 μM AS101, with or without 1000 units/ml catalase for 7 h and then stained with JC-1 to test MMP. (B) Cells given the same treatments but for 11 h were analyzed for expression of the activated form of caspase-9 as described under Section 2. The results show one representative experiment of three performed. (C) Detection of apoptotic cells by annexin V and PI staining for cells treated with 0.64 or 1.6 μM AS101 and 100 μM cysteamine with or without 1000 units/ml catalase for 24 h.

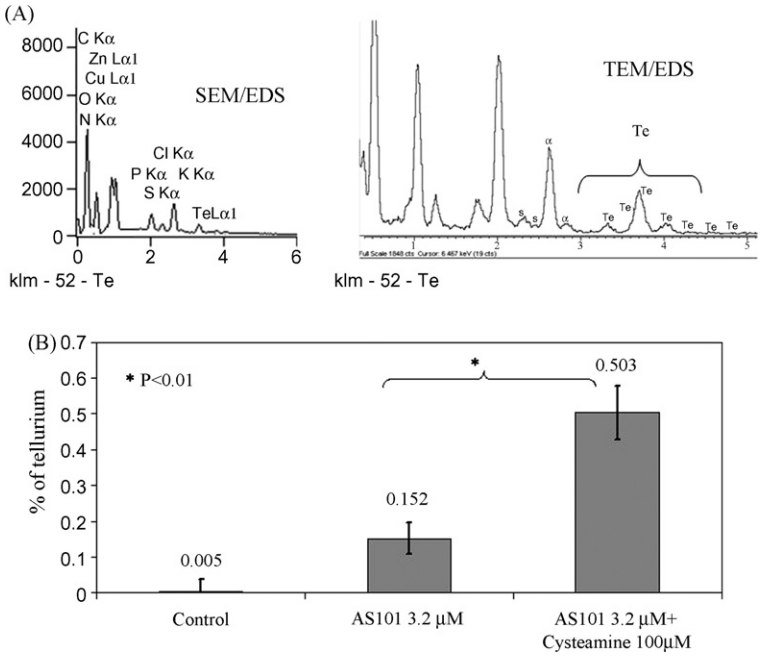

Fig. 6 – Cysteamine elevates the level of tellurium within the Jurkat cells. (A) Cells were treated with 3.2 μM AS101 and 100 μM cysteamine for 4 h, fixed in ethanol, dripped on to a carbon grid and analyzed with the SEM/EDS and TEM/EDS. (B) Cells were treated with AS101 and cysteamine and the percentage of tellurium within the cells was detected with TEM/EDS. Data represent mean ± S.E. from three independent experiments.

levels in cells treated with AS101 and cysteamine (data not shown). Thus, we suggest that AS101 together with cysteamine induce an increase in ROS, which then leads to a disruption of the mitochondrial membrane potential and finally to the activation of caspase-9.

3.4. *Cysteamine elevates the level of tellurium within the Jurkat cells*

2-Mercaptoethanol interacts with cystine to form a mixed disulfide, which is taken up by the cells mainly via the L system, a transport system for neutral α-amino acids such as leucine [15]. We examined whether the interaction between thiols and AS101 enables the AS101 to be more readily taken up by the cells. We used energy dispersive X-ray spectroscopy (EDS) to evaluate the presence of tellurium within the cells. Fig. 6a shows that both the SEM/EDS and the TEM/EDS detected an emission line specific for tellurium in cells grown with AS101 and cysteamine. No such peak was detected in the untreated control (data not shown). Next, we quantified the percentage of tellurium within the Jurkat cells, with and without the addition of cysteamine. The results show that cysteamine significantly elevated, by a 3.5-fold, the percentage of tellurium within the cells in comparison to those grown with AS101 alone (Fig. 6b). When comparing the control cells to those with AS101 alone, a small increase was observed; however the values are in the instrument's range of uncertainty and therefore cannot be considered significant. The results suggest that the reaction between the thiol and AS101 yields a compound that is more readily taken up by the cells.

3.5. *The origin of thiol selectivity in potentiating AS101 activity*

We have shown that the compound that is probably formed from the reaction between AS101 and cysteamine is readily incorporated through the cell membrane and once inside induces the malignant T cells to undergo apoptosis. This however did not occur with some other thiols like cysteine and GSH. Further analysis revealed that the thiols that do not potentiate the effect of AS101 have a free carboxyl and amine. We speculated that charges on the compound formed by the interaction between AS101 and these thiols might inhibit uptake through the cell membrane. In order to test this hypothesis, we used two compounds; L-cysteine methyl ester (CYSM) and *N*-acetyl-L-cysteine (NAC). CYSM does not have a free carboxylate and therefore could not carry a negative charge. In NAC the amino group is acetylated, and therefore does not carry a positive charge. Fig. 7 shows that AS101 together with CYSM, but not with NAC, caused an increase in apoptosis in Jurkat cells. Furthermore, tellurium could be detected in cells grown with CYSM but not with NAC (data not shown). These results suggest that the negative charge on the carboxylate inhibits the entry of the AS101 thiol product into the cell.

4. Discussion

In this study we present evidence that the synthetic Te(IV) compound AS101 increases the accumulation of cells in the G_2/M phase of the cell cycle. We show that the addition of

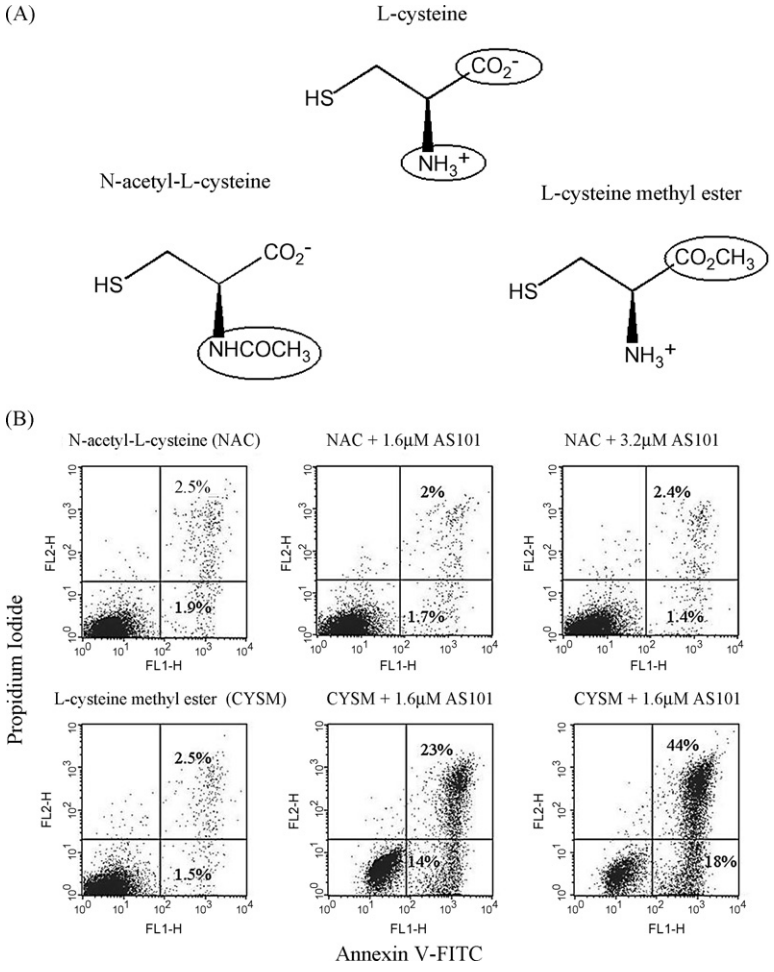

Fig. 7 – Understanding the interaction between thiols and AS101. (A) Chemical structure of L-cysteine, L-cysteine methyl ester and N-acetyl-L-cysteine. (B) Jurkat cells were treated with AS101 together with L-cysteine methyl ester or N-acetyl-L-cysteine (100 μM) for 24 h. Cell death was assessed by FACScan analysis of annexin-V–FITC/PI staining. The results show one representative experiment of three performed.

thiols that do not contain a free carboxylate can synergistically increase apoptosis, which is accompanied by an increase of ROS, a decrease of the mitochondrial membrane potential and an increase of the percentage of cells that express the initiator caspase (caspase-9) and the effector caspase (caspase-3). Finally we show that certain thiols increase the tellurium concentration within the cells.

Selenium, an essential nutritional trace element, is positioned in group 16 of the periodic table of elements, directly above tellurium, and resembles it in its chemical activity and physical properties. Many of selenium's attributes are similar to those of tellurium and it was shown to be effective in preventing the initiation of cancer at supranutritional levels of dietary intake in animals and humans [24]. Various selenium compounds can react with thiols to either form adducts or modify the original compounds and change their chemical or biological activities. The toxic effect of selenite (Se^{4+}), one of the most extensively investigated selenium compounds, on tumor cells is enhanced by the addition of glutathione [25]. Furthermore, the reaction of some thiols such as cysteamine and cysteine with selenite results in selenium compounds that are rapidly absorbed by the intestinal epithelium [17]. About 65 years ago, Painter proposed that the toxicity of inorganic selenium could be related to the oxidation of thiols of biological importance [26]. He proposed that selenite readily oxidizes sulfhydryl groups, producing disulfide and an unstable selenotrisulfide intermediate, which subsequently may decompose to elemental selenium. Several years later, Ganther [27] accomplished the isolation of selenotrisulfide in a pure form. Similarly, tellurium(IV) also interacts with thiols, producing disulfide and $Te^{II}(thiol)_2$ products. The latter can further react to form a second disulfide and elemental tellurium. We have shown that although AS101 can react with many thiols [8], it produces a synergistic apoptotic effect only with 2-ME and cysteamine, thiols that do not contain a free carboxylate (Fig. 2). Thus, we speculate that only a tellurium-thiol compound that does not contain a free carboxylate can be readily taken up by the cells.

Our results in Fig. 6 show that the thiols increase both the level of apoptosis and the level of tellurium within the cells 3.5-fold. If the role of the thiols is only to increase the level of the intracellular tellurium this would suggest that high

dosages of AS101 alone should show a similar apoptotic effect as low dosages together with the thiols. However the results in Fig. 1 show that even at relatively high concentrations of AS101 no apoptosis was detected. We speculate that since the concentration detected by the TEM/EDS for AS101 alone was within the instruments range of uncertainty its true concentration might be a great deal lower and therefore the difference between the cells uptake of tellurium with and without the thiols might be far greater than a 3.5-fold. In addition the apoptotic effect might be due not only to an increase in the level of intracellular tellurium but that the combination of AS101 and cysteamine or 2-ME might in itself induce a different apoptotic effect.

Cells possess antioxidant systems to control the redox state, which is important for their survival. Excessive production of ROS gives rise to the activation of events that can lead to death in several cell types [28]. In our study we demonstrated, that AS101 and thiols increase the level of oxidative stress within the cells in a dose dependent manner. Mitochondrial dysfunction was detected a little later than the oxidative stress, followed by the activation of the caspase enzymes. The antioxidant catalase significantly reduced the apoptosis. These results suggest that ROS plays an important role during the induction of apoptosis by AS101 and the thiols. In addition to catalase, we tested the effect of *N*-acetylcysteine (NAC) and glutathione (GSH), which were both shown to exhibit antioxidant activity [29,30]. We found that they were both able to significantly reduce the apoptosis induced by AS101 and cysteamine (data not shown). However we speculate that this inhibition might have been due to the direct interaction of their thiols with AS101 which obstructed its interaction with cysteamine and not because of their cellular antioxidant activity.

In order to compare the above results to the effect of AS101 together with the thiols on primary cells, PBMCs grown in human serum were incubated with AS101 with and without 2-ME or cysteamine for long periods of time, but no significant effect was detected either in the cell cycle or in the level of apoptosis of the cells (data not shown). Membrane transporters play an important role in mediating chemosensitivity and resistance of tumor cells [31]. Amino acid transporters play a role in drug sensitivity, by mediating the uptake of amino acid analog drugs, but also in drug resistance, promoting tumor growth by providing essential amino acids. SLC7A5, encoding the sodium-independent L-type amino acid transporter 1 (LAT1), transports large, branched-chain and aromatic, neutral amino acids, including several essential amino acids. SLC7A5 displays broad substrate selectivity for amino acid-like compounds such as melphalan [32,33]. In preliminary results we have found that BCH (2-aminobicycloheptane-2-carboxylic acid), an L-system inhibitor, can significantly reduce the apoptotic effect of AS101 and cysteamine (data not shown). We speculate that the L-system might play a role in the absorption of AS101 or the product of its interaction with thiols into the cells. In addition, its higher expression in malignant cells might explain the difference in the effect of AS101 and the thiols on the Jurkat cells in comparison to the human PBMCs. More research is required in order to characterize the compound or compounds that are formed in the reaction between AS101 and the thiols and to understand the mechanism by which they are incorporated into the tumor cells.

The area of tellurium and organotellurium chemistry was slow to develop, but in the last 30 years, thousands of studies investigating various aspects of these compounds were published. Nevertheless, little is known with regard to its biological and pharmacological effects [9]. This is probably due to the fact that no specific biological function for tellurium has been identified [34], and it is still considered a non-essential element for life. On the other hand, selenium is an essential trace element whose necessity was also not recognized, until the discovery of selenium-dependent enzymes such as glutathione peroxidase [35]. Following this interest in selenium and the introduction by us and others of some synthetic tellurium compounds [36], we anticipate a similar increase in the applications of tellurium derivatives in biology and biochemistry.

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