BIOCHEMICAL PHARMACOLOGY 72 (2006) 1423–1431

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

The immunomodulator AS101 induces growth arrest and apoptosis in Multiple Myeloma: Association with the Akt/Survivin pathway

Michal Hayun [a], *Yaniv Naor* [a], *Merav Weil* [a], *Michael Albeck* [b], *Alpha Peled* [c], *Jeremy Don* [a], *Nechama Haran-Ghera* [c], *Benjamin Sredni* [a,*]

[a] *Safdié Institute for AIDS and Immunology Research, The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel*

[b] *Department of Chemistry, Bar-Ilan University, Ramat-Gan, Israel*

[c] *Department of Immunology, The Weizmann Institute of Science, Rehovot, Israel*

ARTICLE INFO

Article history:
Received 30 April 2006
Accepted 13 June 2006

JEL classification:
Inflammation
Immunopharmacology

Keywords:
AS101
Multiple Myeloma
Survivin
Akt
Apoptosis
Caspases

Abbreviations:
Cdk, cyclin-dependent kinase
IAPs, inhibitors of apoptosis proteins
IGF-1, insulin-like growth factor 1
MM, Multiple Myeloma
PI, propidium iodide
PI3-K, phosphatidylinositol-3-OH kinase

ABSTRACT

Multiple Myeloma (MM) is a clonal B-cell malignancy affecting both the immune and the skeletal systems, and accounts for 10% of all hematological cancers. The immunomodulator ammonium trichloro (dioxoethylene-O,O′) tellurate (AS101) is a non-toxic compound which has direct anti-tumoral properties in several tumor models. The present study examined the anti-tumoral activity of AS101 in MM by targeting the Akt/Survivin signaling pathway, crucial for survival. We showed that AS101 inhibites cell proliferation and colonies formation of MM cell lines, in a dose-dependent manner. AS101 induced G_2/M growth arrest and increased both cyclin-dependent kinase inhibitor $p21^{waf1}$ protein levels and Cdk1 ($p34^{cdc2}$)—inhibitory phosphorylation. Longer incubation of MM cells with AS101 resulted in accumulation of apoptotic cell population and in increased caspase 9, 3 and 7 activities. We also showed that AS101 down-regulated Akt phosphorylation and decreased expression of the inhibitor of apoptosis, survivin. Since Akt and survivin are potentials targets for MM therapy, we suggest that AS101, currently being used in clinical studies, may have therapeutic implications in myeloma and other hematopoietic malignancies.

* *Corresponding author*. Tel.: +972 3531 8250; fax: +972 3635 6041.
E-mail address: srednib@mail.biu.ac.il (B. Sredni).

0006-2952/$ – see front matter
doi:10.1016/j.bcp.2006.06.015

1. Introduction

Multiple Myeloma (MM) is an incurable hematological malignancy of differentiated B lymphocytes characterized by accumulation of clonal plasma cells in the bone-marrow [1,2]. The main clinical manifestations of the disease include pancytopenia, hyperproteinemia, renal dysfunction, bone lesions and immunodeficiency [2–4]. Although patients suffering from MM may initially respond to chemotherapy, they ultimately become resistant to such a therapy. Only 5% of patients achieve complete remission, and the median survival is 30–36 months [5,6]. Therefore, more effective and less toxic treatment options are needed in the battle against MM.

Multiple signaling cascades, including the Janus family tyrosine kinase (JAK)/signal transducer and activator of transcription (STAT) proteins, the Ras/Raf/Mek/Erk and the phosphatidylinositol-3-OH kinase (PI3-K)/Akt pathways, are activated in MM [2,7]. The PI3-K/Akt pathway is of particular interest because of its role in inhibiting apoptosis and promoting cell proliferation [8]. In Multiple Myeloma, insulin like growth factor-1 (IGF-1) activates PI3-K/Akt pathway, leading to both proliferative and anti-apoptotic effects [9].

The inhibitors of apoptosis proteins (IAPs) are a family of intracellular anti-apoptotic proteins that play a key role in cell survival by modulating death-signaling pathways at the post-mitochondrial level [10]. Survivin is a member of the IAPs proteins, which becomes the fourth most expressed transcript in human cancer, but not in normal tissues [10,11]. It has dual activity as inhibitor of apoptosis and as regulator of cell cycle [12]. Recent studies have shown that survivin has the ability to inhibit the key molecules of the apoptotic machinery, the caspases and is a downstream target in both JAK/STAT and PI3-K/Akt pathways [13–15].

The non-toxic immunomodulator Ammonium trichloro (dioxoethylene-O,O′) tellurate (AS101), first developed by us [16], is a low molecular weight organic tellurium compound (Fig. 1A). AS101 possess immunomodulating properties and have beneficial effects in diverse pre-clinical and clinical studies. In a variety of tumor models, AS101 has been found to have a clear anti-tumor properties [16,17]. AS101 was shown to improve the survival of Madison lung carcinoma-bearing mice when given in combination with chemotherapy [18]. In another study, combined treatment of AS101 with low doses of paclitaxel (Taxol) enhanced survival of B16 melanoma tumor-bearing mice by up-regulating Fas/Apo-1 expression [19].

Phase I clinical trials on advanced cancer patients treated with AS101 showed increased production and secretion of a variety of cytokines, leading to a clear dominance in the Th1 response with a decrease in the Th2 response [20]. Phase II 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 chemotherapy [17,21].

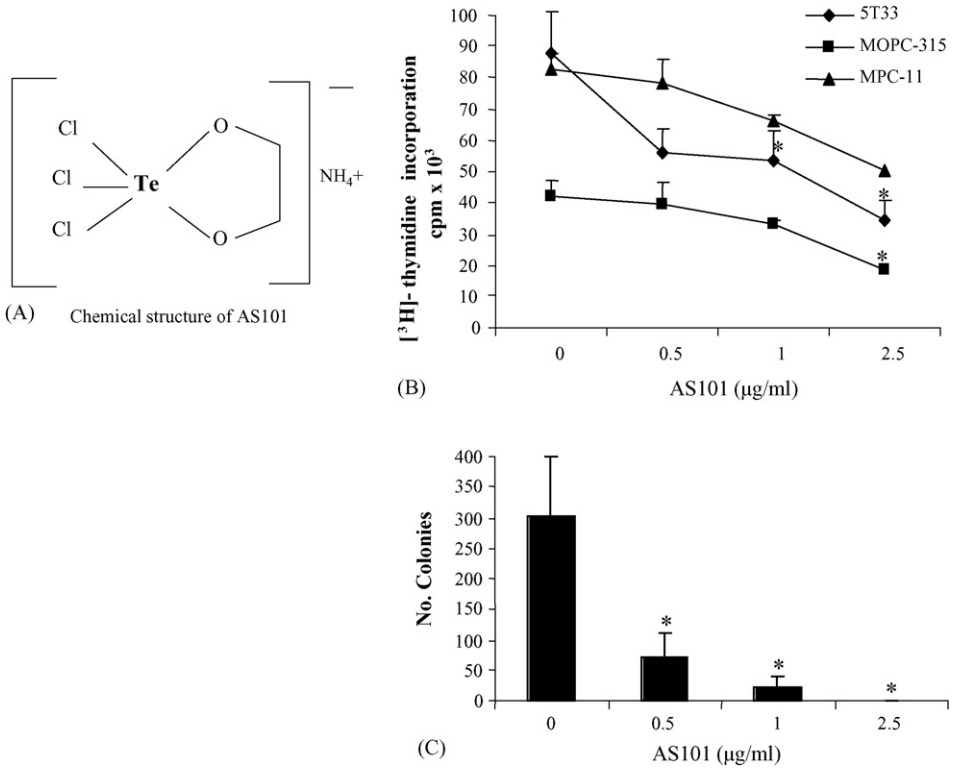

Fig. 1 – Inhibitory effect of AS101 on MM cell proliferation and colonies formation. 5T33 (0.5×10^6/ml), MOPC-315 (0.5×10^6/ml) and MPC-11 (0.25×10^6/ml) MM cells were cultured with AS101 (0.5–2.5 μg/ml) for 48 h. The ability of the MM cells to proliferate was measured by [^{3}H] thymidine uptake assay (B). 5T33 (10^4 cells/plate) were seeded in soft agar culture in the presence of AS101 (0.5–2.5 μg/ml). Colonies were scored after 10–12 days of incubation (C). Results represent mean ± S.D. from four independent experiments. $^*p \leq 0.05$ decrease vs. untreated cells.

Most of AS101 activities have been primarily attributed to the direct inhibition of the anti-inflammatory cytokine IL-10 [22]. Additionally, AS101 was found to interfere in cell cycle regulation and also to induce apoptosis cell death, in several studies [23,24].

In our previous studies, the activities of AS101 in different tumor models, was concentrated in its ability to modulate cytokines. This study examined the direct anti-tumoral activity of AS101 in MM cells and its mechanism of action. Our results indicated that AS101 induces G_2/M growth arrest and apoptosis of the myeloma cells by up-regulating Cdk1-inhibitory phosphorylation and down-regulating survivin expression, in association with the Akt pathway.

2. Materials and methods

2.1. Cell culture

Mouse MM cell lines (5T33, MPC-11 and MOPC-315) were generously provided by Prof. Haran-Ghera from Weizmann Institute, Israel. The cells were grown in RPMI-1640 medium with 10–15% fetal calf serum supplemented with 1 mM sodium pyruvate, 2 mM L-glutamine and 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. The culture cells were over-night seeded prior to all experiments.

2.2. Compounds and antibodies

AS101 was supplied by M. Albeck from Bar-Ilan University, Israel, in a solution of PBS (pH 7.4) and maintained at 4 °C. Antibodies for Western blotting: anti-$p21^{waf1}$, anti-Cdk1, anti-pAkt (detects phosphorylated Ser^{473}), anti-Akt and anti-α-Tubulin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA); anti-phosphorylated Cdk1 ($pT^{14}pY^{15}$) (Biosource, Invitrogen, USA), and anti-β-Actin (Sigma, St. Louis, MO). Recombinant IGF-1 was obtained from Cytolab (Pepro-Tech. ink, NJ).

2.3. Proliferation assay

Cell proliferation was measured by adding 0.4 μCi/mM [^{3}H]-thymidine (Sigma, St. Louis, MO) per well of a 96-well plate (2×10^4 cells/200 μl), 24 h prior to cells harvesting. The [^{3}H]-thymidine incorporation was measured by liquid scintillation counting (TOP Counter NXT, Packard).

2.4. Clonogenic assay

The soft agar method, described by Pluznik and Sachs [25], based on the preparation of two layers of agar at different concentrations, has been used: AS101 was incorporated into 2 ml of hard agar medium in a 35 mm Petri dish. The 5T33 cells (1×10^4) in 1 ml of soft agar medium were cloned above the hard agar. After 10–12 days of incubation at 37 °C, the colonies were identified and counted (colonies with >50 cells were counted) using an inverted binocular microscope.

2.5. Cell cycle distribution studies

Culture cells were rinsed with PBS (Ca^{2+} and Mg^{2+} free) and suspended in the dark for 30 min at 4 °C in 0.5 ml buffer, containing 50 μg/ml propidium iodide (PI), 0.1% sodium citrate, 0.1% Triton-X and 1 mg/ml RNase. DNA content was measured using a FACStar plus (Becton Dickinson, San Jose, CA) flow cytometer using Cell Quest software.

2.6. Detection of apoptosis

Determination of cells undergoing apoptosis was assessed by double staining for Annexin V/PI using an apoptosis detection kit (Bender Med Systems Inc., USA). Cultured myeloma cells were collected and washed with cold PBS (Ca^{2+} and Mg^{2+} free), re-suspended in binding buffer with Fluorescien conjugated Annexin-V and PI, incubated in dark for 15 min and then analyzed by flow cytometry using Cell Quest software.

Identification of different cell populations: vital cells (PI^-/$Annexin^-$); early apoptotic cells (PI^-/$Annexin^+$); cells undergoing late apoptosis (PI^+/$Annexin^+$).

2.7. Detection of caspase activity

Caspase activation assay was performed using Fluorescein Caspase Activity Kit (Alexis Biochemicals, San-Diego, CA). This kit detects active caspase in living cells utilizing unique (carboxyfluorescein) chemistry; the fluorochrome caspase inhibitor binds covalently to the active site of the caspase enzyme. In brief, FLICA solution (30×) was adding to 300 μl (1×10^6/ml) of cell suspension and incubated for 1 h at 37 °C. The samples were washed with wash buffer and the suspended cells were analyzed by flow cytometry (FL-1 channel) using an argon ion laser at 488 nm.

2.8. Western blot analysis

Cell extracts were prepared by suspension in ice-cold lysis buffer containing 20 mM Tris (pH 7.4), 150 mM NaCl, 1mM EDTA, 1% Triton X-100, 4 mM $NaVO_4$, 1 mM PMSF, 5 μg/ml aprotonin and 5 μg/ml leupeptin. Next, 20–30 μg of cell lysates were subjected on 10–15% SDS-PAGE gel. Following electrophoresis, the gels were transferred to nitrocellulose membrane, immersed in blocking solution (5% dry milk in TBST buffer for 1 h) and incubated with primary antibody (overnight, 4 °C). The blot was washed with TBST buffer containing 0.1% Tween-20, incubated for 1 h with HRP-conjugated secondary Abs, and rewashed. Proteins were visualized using the enhanced chemiluminescence detection system (ECL; Pierce biotech, USA).

2.9. Statistical analysis

Values are expressed as mean ± S.D. Statistical significance between treated cells and controls was determined by using 2-tails Student's *t*-test. Significance was established at a value of $p < 0.05$.

3. Results

3.1. *Effect of AS101 on MM cell proliferation and colonies formation*

Previous studies have shown that AS101 has an anti-proliferative effect on different tumor cell lines, which was also reflected in the reduction in their colony formation on soft-agar. Based on these data, the anti-proliferative effect of AS101 was examined in MM cell lines. As can be seen in Fig. 1B, AS101 inhibited 5T33, MOPC-315 and MPC-11 cells proliferation, in a dose-dependent manner. Maximal decrease of 2.5-fold in 5T33, 2.2-fold decrease in MOPC-315, and 1.7-fold decrease in MPC-11 cell proliferation were observed at concentration of 2.5 μg/ml AS101. The ability of 5T33 cells to form colonies on soft agar was effectively reduced up to complete inhibition by AS101 at concentration of 2.5 μg/ml (Fig. 1C). These results suggest that AS101 has anti-proliferate activity on MM cells that can be partly explained by a direct inhibitory effect as reflected in the reduction of 5T33 cells colony formation.

3.2. *AS101 induces G_2/M arrest in MM cell lines*

We aimed to determine whether the inhibitory effect of AS101 on MM cell proliferation, is mediated through alterations of the cell cycle progression. Cell cycle progression was assessed in 5T33, MPC-11 and MOPC-315 cells exposed to AS101 (1, 2.5 μg/ml) for 48 h. As can be seen in Fig. 2A, treatment of the

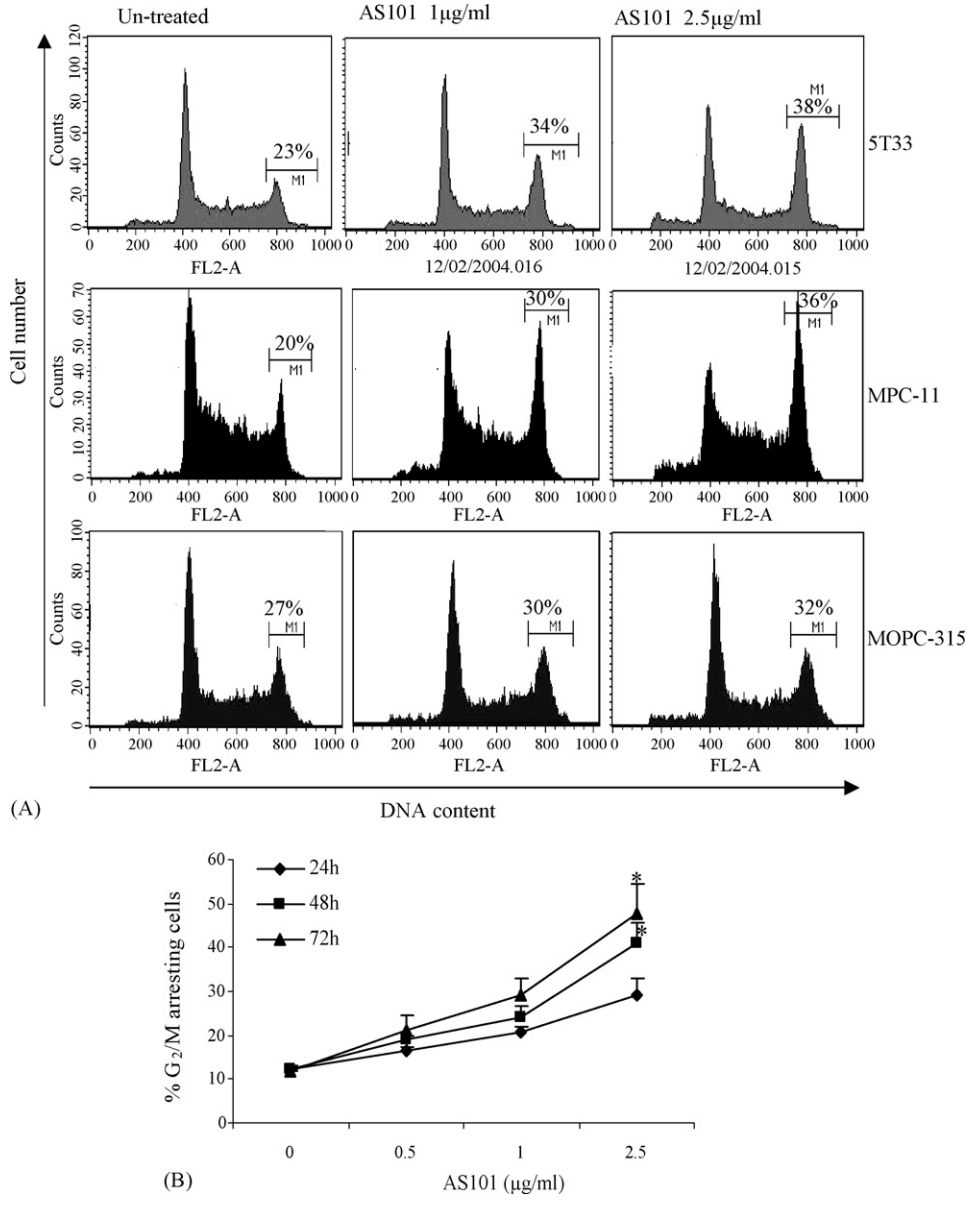

Fig. 2 – Treatment of MM cells with AS101 results in increased accumulation of cells in G_2/M phase. Cell cycle distribution was assessed in 5T33, MPC-11 and MOPC-315 MM cells exposed to 1 or 2.5 μg/ml AS101, for 48 h (A). 5T33 (0.5×10^6/ml) were incubated with AS101 (0.5–2.5 μg/ml) at 24, 48 and 72 h (B). 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 (A) and mean ± S.D. from three independent experiments (B). $^*p < 0.02$ increase vs. untreated cells.

above myeloma cells with AS101 resulted in a shift from G_1 to G_2/M phase, with accumulation of cells in the G_2/M phase, in a dose-dependent manner. Similar results were observed for the human U266 and RPMI 8226 MM cells (data not shown). Exposure of 5T33 cells to AS101 resulted in a dose- and time-dependent increase in the G_2/M phase population (Fig. 2B). Significant accumulation of 5T33 cells, 38% at 48 h and 44% at 72 h, was observed following incubation with 2.5 μg/ml AS101. Similar increase in G_2/M phase was also achieved as soon as 24 h of incubation, with a higher dose of AS101 (10 μg/ml).

3.3. *Induction of apoptosis by AS101 in MM cell lines*

Long-term exposure to AS101 resulted in an increase of myeloma apoptotic cell death. Apoptosis was quantified by using Annexin-V/PI staining. As can be seen in Fig. 3A, AS101 markedly increased the fraction of apoptotic cells in 5T33,

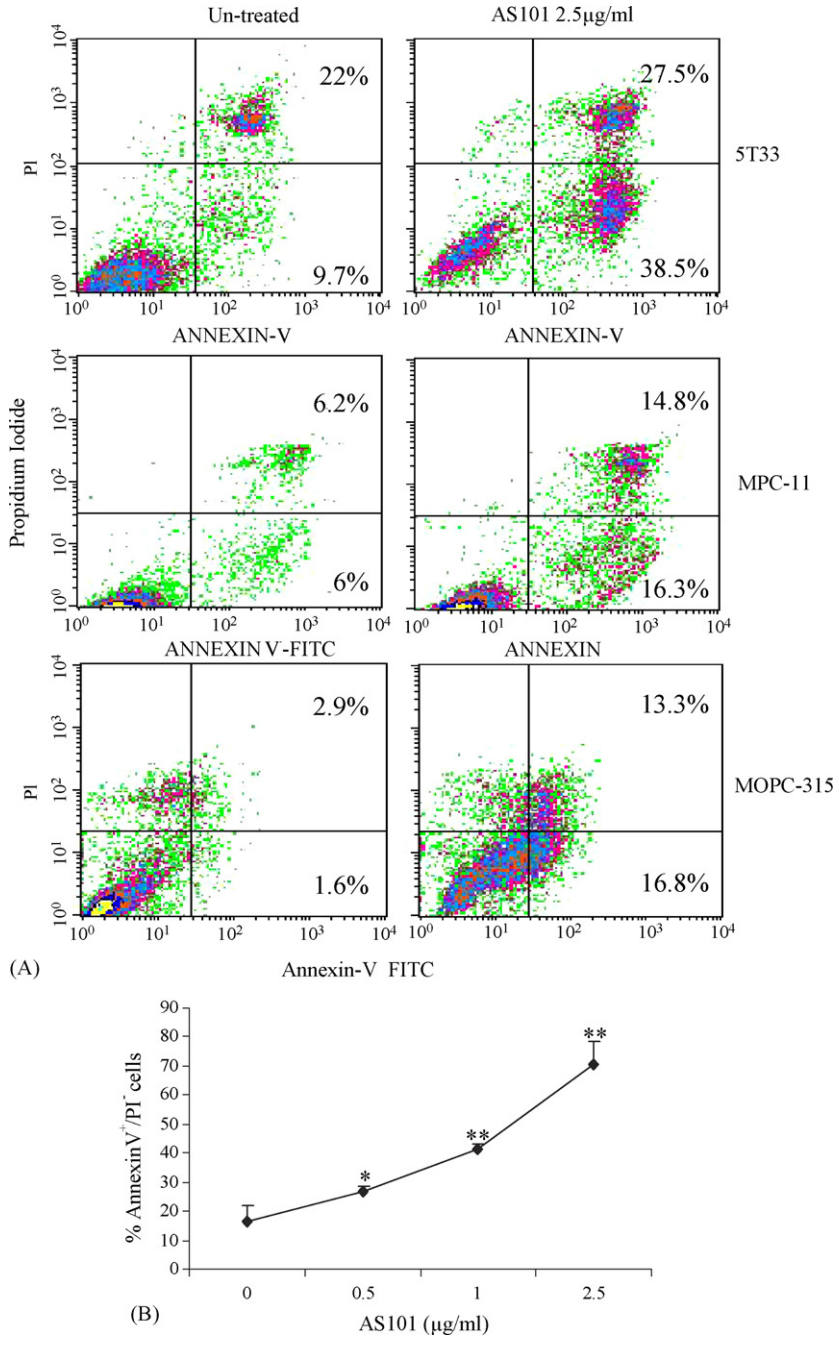

Fig. 3 – Induction of Apoptosis by AS101 treatment. 5T33, MPC-11 and MOPC-315 cells (0.25×10^6/ml) were incubated with AS101 for 72 h (A) or 96 h (5T33 cells) (B). Apoptosis was detected by double staining of the cells with FITC-Annexin V and PI. The percentage of apoptotic cells (early and late apoptosis) was estimated by flow cytometry analysis. Results show one representative experiment out of three performed (A) or mean ± S.D. from three independent experiments (B). $^{*}p < 0.05$ or $^{**}p < 0.01$, increases vs. untreated cells.

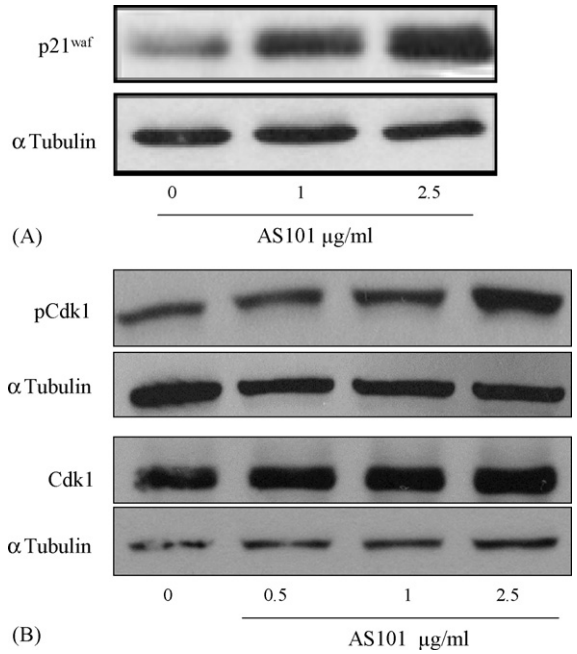

Fig. 4 – Up-regulation of regulatory proteins involved in cell cycle control by AS101. Lysates were obtained from 5T33 cells treated for 48 h with 1 or 2.5 μg/ml AS101 (A) or with 0.5–2.5 μg/ml AS101 for 24 h (B). Western blot analysis was performed by using anti-p21^{waf1}, anti- α-Tubulin, anti-Cdk1 or anti-Cdk1 (pT^{14}pY^{15}) antibodies. Results show one representative experiment out of three performed.

MOPC-315 and MPC-11 cells following 72 h of incubation. The 5T33 cells demonstrated the highest sensitivity among all cell types; they showed increase of 34.3% in early and late apoptotic cells versus un-treated cells. The MOPC-315 and MPC-11 cells showed 25.6 and 19% increase in apoptotic cells, respectively (Fig. 3A). Analysis of 5T33 cells undergoing early apoptosis induced by incubation with AS101 for 96 h is presented in Fig. 3B. In this cell line, AS101 increased apoptosis in a dose-dependent manner and reached 70% of early apoptotic cells, by using 2.5 μg/ml (Fig. 3B). These results suggest that the cells were undergoing apoptosis following the mitotic blockage-induced by AS101.

3.4. *Involvement of AS101 in the G_2/M transition in 5T33 cells*

In order to elucidate the mechanism of action of AS101 in MM, we choose the 5T33 cell line which is unique in its ability to migrate to bone-marrow compartment in vivo, thus mimicking the disease in human, and examined key factors in the G_2/M transition. We found that AS101 up-regulated the protein levels of the Cdk inhibitor p21^{waf1}, a major transcriptional target of p53, in a dose-dependent manner (Fig. 4A). p21^{waf1} is known to be involved in the regulation of G_2/M transition and therefore can inhibit Cdk1 (p34^{cdc2}) activity, which is essential for the entry into mitosis [26]. Treatment of 5T33 cells with AS101 resulted in increased Cdk1 phosphorylation at Thr14 and Tyr15. This phosphorylation causes its inactivation, and thus inhibits the cells to advance from G_2 phase to mitosis (Fig. 4B). Cdk1 protein levels remains un-affected following AS101 treatment (Fig. 4B). These results suggest that AS101 up-regulates p21^{waf1} protein levels or prevents its degradation which in turn leads to Cdk1 inactivation, resulting in arrest of the myeloma cells in the G_2/M phase.

3.5. *Decrease Akt activation, survivin expression and up-regulation of caspase activity -are induced by AS101*

One of the most important survival-signaling pathways is mediated by PI3-K and its downstream target, Akt [27]. We evaluated the activity of this survival protein in response to AS101 exposure in MM cells. Fig. 5A shows that AS101 inhibited the activation of Akt in 5T33 cells, as presented by reduced expression of phosphorylated Akt (Ser^{473}). Akt is known to activate pro-survival genes, among them survivin, which is a main survival factor in many cancer cells [11,28]. For this reason, this anti-apoptotic protein was examined. We found that 5T33 cells express high level of survivin. Treatment of the cells with AS101 resulted in a decrease survivin expression following 24 and 48 h of incubation (Fig. 5B). Survivin directly binds and inhibits caspases 3, 7 and 9 [13]. We therefore examined the activity of these caspases in response to AS101 exposure. As illustrated in Fig. 5(C and D), exposure of 5T33 cells to different concentration of AS101 resulted in a significant up-regulation of caspases 9, 3 and 7 activity, in a dose- and time-dependent manner.

IGF-1 is a growth and survival factor for MM cells and recently reported to promote migration of 5T2 myeloma cells [29]. IGF-1 activates Akt, leading to apoptosis inhibition [30]. Therefore, we examined whether exogenously added recombinant IGF-1 could affect survivin expression. As shown in Fig. 5E, rIGF-1 significantly increased survivin protein level, while addition of AS101 to rIGF-1 pre-treated cultured cell, down-regulated survivin expression level. These data indicate that AS101 might mediate its activity via decrease of Akt activation and survivin protein, thus leading to caspases activation and cellular apoptosis.

4. Discussion

Multiple Myeloma, a malignant proliferation of plasma cells, is requiring new therapeutic strategies. Inhibition of cell cycle progression is considered as a potential therapy for various cancers [31]. Many anticancer agents disrupt the normal cell cycle dynamics, causing arrest in various phases of the cell cycle, which increases tumor cell's sensitivity to apoptosis-inducing agents. This study provides evidence that the non-toxic organic-tellurium compound, AS101, itself, can inhibit growth and induce apoptosis of MM cell lines. Our finding demonstrate that AS101 exerts its activity by interruption with the Akt/Survivin signaling pathway, through mediating G_2/M arrest regulatory proteins, down-regulation of survivin expression and induction of caspases activation.

In this study, we first showed that AS101 acts directly to inhibit the growth of MM cells in a dose-dependent manner, assessed by thymidine-uptake assay and colonies formation. A previous study designed by us showed that AS101 interferes in cell cycle regulation, as demonstrated in the synergistic

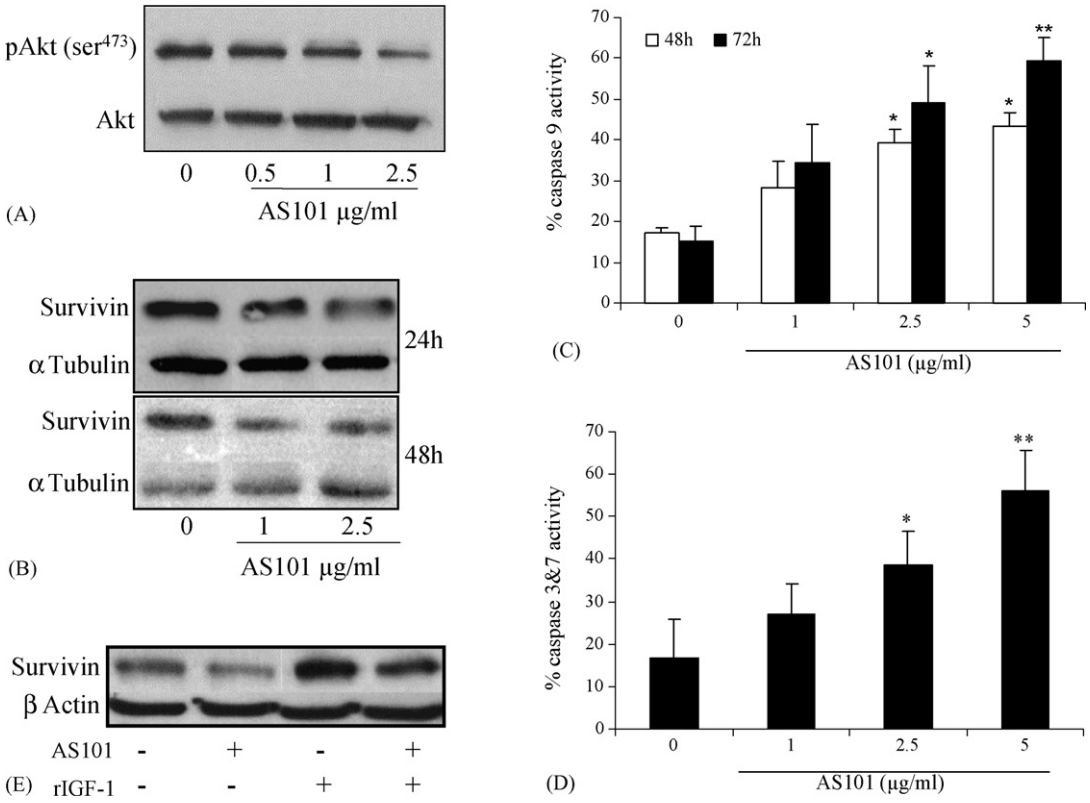

Fig. 5 – AS101 down-regulates survivin expression and induces caspase activation in MM cells. 5T33 cells (0.5×10^6/ml) were incubated with AS101 for 48 h (A), for 24 and 48 h (B) or were pre-incubated for 2 h with 50 ng/ml rIGF-1 and then 2.5 μg/ml AS101 was added to the relevant cultures for a farther 24 h (E). Western blot analysis was performed by using anti-pAkt, anti-Akt, anti-survivin, anti-α-Tubulin and anti-β-Actin. Results show one representative experiment out of three performed (A, B, E). 5T33 cells (0.25×10^6/ml) were incubated with AS101 (1–5 μg/ml) for 48 and 72 h (C) or 72 h (D). Caspase 9 (C) and caspase 3 and 7 (D) activities were measured by using carboxyfluorescein FLICA assay. The results represent mean ± S.D. from three different experiments. $^{**}p < 0.05$ and $^{*}p < 0.01$ are increased vs. untreated cells.

effect of AS101 with PMA in inducing G_1 arrest of human myeloid leukemia cells (HL-60), and thus induced their final differentiation [23]. In addition, via modulations in Cdk inhibitor, AS101 induced G_1 arrest followed by apoptosis of NIH/Ha-Ras transformed cells [24]. That raised the possibility that the growth inhibition induced by AS101 in MM may interfere with cell cycle arrest. We found that AS101 induced G_2/M arrest following 48 h of incubation, in a dose- and time-dependent manner, in three different MM cell lines. Treatment of the myeloma cells with AS101 for 72 and 96 h resulted in increase accumulation of apoptotic cells population. This raised the possibility that AS101 induces transient arrest, forcing the cells to undergo apoptosis. To further elucidate the mechanism of AS101 on apoptosis and G_2/M cell cycle arrest, we studied the cellular protein involved in G_2 checkpoint. Cdk1 is negatively regulated by phosphorylation on the amino acid residue Thr14 and Tyr15 [32] and is inhibited by one of the transcriptional targets of p53, the p21 ^{waf1} protein [33]. Treatment of 5T33 cells with AS101, markedly enhanced $p21^{waf1}$ protein expression. Indeed, incubation of 5T33 cells with AS101 increased cdk1 phosphorylation resulting in its inactivation. This is consistent with Fukuda et al. who found that the phosphorylation of Cdkl at Tyr15 is lower in $p21^{-/-}$ bone-marrow cells [34].

In recent years, considerable efforts have been made to develop strategies for modulating apoptosis in cancer and other human diseases [35]. In this context, approaches to counteract survivin in tumor cells have been proposed with the dual aim to inhibit tumor growth through an increase in spontaneous apoptosis, and to enhance tumor cell response to apoptosis-inducing agents [36]. Survivin is regulated in a highly cell cycle dependent manner, with a marked increase in the G_2/M phase [14]. During this phase survivin associates with and is phosphorylated by $p34^{cdc2}$/cyclin B_1 kinase [37,38]. Because AS101 induced G_2/M arrest and also decreased pCdk1 activity, it was tempting to find-out if it can reduce survivin protein levels. We could note that within 24 h of AS101 treated 5T33 cells, Cdk1 phosphorylation was up-regulated (Fig. 4B), followed by down-regulation of survivin (Fig. 5B). Down-regulation of survivin has recently been demonstrated in MM cells treated with various nuclear factor-kappaB inhibitors [39]. Targeting survivin by means of different approaches demonstrated that inhibition of this cytoprotective factor was able to promote spontaneous apoptosis in tumor cells [36,38]. The ability of AS101 to induce apoptosis in MM cells might be therefore, due to its ability to reduce survivin levels, which permits caspases activation.

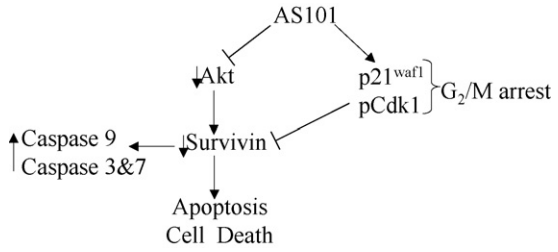

Fig. 6 – Schematic illustration of AS101 mechanism of action in inducing growth arrest and apoptosis in MM cells. Akt/Survivin pathway mediates cell growth and survival in MM. Using AS101 compound, Akt activity is suppressed in one hand, and on the other hand, AS101 arrest cells in G_2/M phase, through up-regulation of $p21^{waf1}$ and pCdk1, which in turn reduced survivin levels, leading to activation of caspases and finally induces myeloma cell death.

Survivin is a downstream target in both JAK/STAT and PI3-K/Akt pathways [13–15]. We found that 5T33 cells do not express constitutively phosphorylated Stat3 (data not shown), therefore, we eliminated the possibility that survivin is down-regulated by AS101 via the Jak/Stat3 pathway, and examined whether that down-regulation is mediated via Akt. Indeed, we found that AS101 down-regulates Akt phosphorylation (ser^{473}) in a dose-dependent manner. This result is supported by the recent findings of Katayama et al., that inactivation of Akt by LY294002 induced G_2/M arrest along with the inhibitory phosphorylation of Cdk1 [40]. Signaling via PI3-K/Akt is initiated by multiple stimuli, especially by IGF-1, in myeloma cells [41]. We showed that AS101 could diminish the effect of exogenously added of rIGF-1 on survivin expression. We have schematically represented a potential mechanism used by AS101 in targeting cell cycle arrest and apoptosis in MM cells (Fig. 6). On the basis of our findings in this study, we initiated an in vivo study with murine 5T33 myeloma bearing mice treated with AS101. Our preliminary results showed prolonged survival of myeloma bearing mice following AS101 treatment.

In summary, AS101 induces growth arrest and apoptosis in different MM cell lines. We suggest that this AS101's activity is exerted via decrease of Akt activation, induction of $p21^{waf1}$ protein and Cdk1-inhibitory phosphorylation-induced G_2/M arrest, leading to reduce survivin levels and apoptosis induction. Our results may have clinical implications in the use of AS101, alone or combined with conventional and novel therapies, in myeloma treatment and possibly in other malignancies.

Acknowledgments

The study was partly supported by the Dave and Florence Muskovitz Chair in Cancer Research, The Frieda Stollman Cancer Memorial Fund and The Dorsha Wallman Cancer Research Endowment.

This study is a part of Michal Hayun's Ph.D.

REFERENCES

[1] Reece DE. New advances in multiple myeloma. Curr Opin Hematol 1998;5:460–4.
[2] Sjak-Shie NN, Vescio RA, Berenson JR. Recent advances in multiple myeloma. Curr Opin Hematol 2000;7:241–6.
[3] Anderson K. Advances in the biology of multiple myeloma: therapeutic applications. Semin Oncol 1999;26:10–22.
[4] Hallek M, Bergsagel PL, Anderson KC. Multiple myeloma: increasing evidence for a multistep transformation process. Blood 1998;91:3–21.
[5] Kastrinakis NG, Gorgoulis VG, Foukas PG, Dimopoulos MA, Kittas C. Molecular aspects of multiple myeloma. Ann Oncol 2000;11:1217–28.
[6] Feinman R, Koury J, Thames M, Barlogie B, Epstein J, Siegel DS. Role of NF-kappaB in the rescue of multiple myeloma cells from glucocorticoid-induced apoptosis by bcl-2. Blood 1999;93:3044–52.
[7] Platanias LC. Map kinase signaling pathways and hematologic malignancies. Blood 2003;101:4667–79.
[8] Datta SR, Brunet A, Greenberg ME. Cellular survival: a play in three Akts. Genes Dev 1999;13:2905–27.
[9] Qiang YW, Kopantzev E, Rudikoff S. Insulinlike growth factor-I signaling in multiple myeloma: downstream elements, functional correlates, and pathway cross-talk. Blood 2002;99:4138–46.
[10] Pallares J, Martinez-Guitarte JL, Dolcet X, Llobet D, Rue M, Palacios J, et al. Survivin expression in endometrial carcinoma: a tissue microarray study with correlation with PTEN and STAT-3. Int J Gynecol Pathol 2005;24:247–53.
[11] Velculescu VE, Madden SL, Zhang L, Lash AE, Yu J, Rago C, et al. Analysis of human transcriptomes. Nat Genet 1999;23:387–8.
[12] Li F, Ambrosini G, Chu EY, Plescia J, Tognin S, Marchisio PC, et al. Control of apoptosis and mitotic spindle checkpoint by survivin. Nature 1998;396:580–4.
[13] McCormick F. Cancer: survival pathways meet their end. Nature 2004;428:267–9.
[14] LaCasse EC, Baird S, Korneluk RG, MacKenzie AE. The inhibitors of apoptosis (IAPs) and their emerging role in cancer. Oncogene 1998;17:3247–59.
[15] Kanda N, Seno H, Konda Y, Marusawa H, Kanai M, Nakajima T, et al. STAT3 is constitutively activated and supports cell survival in association with survivin expression in gastric cancer cells. Oncogene 2004;23:4921–9.
[16] Sredni B, Caspi RR, Klein A, Kalechman Y, Danziger Y, Ben Ya'akov M, et al. A new immunomodulating compound (AS-101) with potential therapeutic application. Nature 1987;330:173–6.
[17] Kalechman Y, Albeck M, Oron M, Sobelman D, Gurwith M, Horwith G, et al. Protective and restorative role of AS101 in combination with chemotherapy. Cancer Res 1991;51:1499–503.
[18] Kalechman Y, Shani A, Dovrat S, Whisnant JK, Mettinger K, Albeck M, et al. The antitumoral effect of the immunomodulator AS101 and paclitaxel (Taxol) in a murine model of lung adenocarcinoma. J Immunol 1996;156:1101–9.
[19] Kalechman Y, Longo DL, Catane R, Shani A, Albeck M, Sredni B. Synergistic anti-tumoral effect of paclitaxel (Taxol)+AS101 in a murine model of B16 melanoma: association with ras-dependent signal-transduction pathways. Int J Cancer 2000;86:281–8.
[20] Sredni B, Tichler T, Shani A, Catane R, Kaufman B, Strassmann G, et al. Predominance of TH1 response in tumor-bearing mice and cancer patients treated with AS101. J Natl Cancer Inst 1996;88:1276–84.

[21] Sredni B, Albeck M, Tichler T, Shani A, Shapira J, Bruderman I, et al. Bone marrow-sparing and prevention of alopecia by AS101 in non-small-cell lung cancer patients treated with carboplatin and etoposide. J Clin Oncol 1995;13:2342–53.
[22] Sredni B, Weil M, Khomenok G, Lebenthal I, Teitz S, Mardor Y, et al. Ammonium trichloro (dioxoethylene-o,o')tellurate (AS101) sensitizes tumors to chemotherapy by inhibiting the tumor interleukin 10 autocrine loop. Cancer Res 2004;64:1843–52.
[23] Bouganim-Hayun M, Kalechman Y, Albeck M, Sredni B. The synergistic activity of AS101 and PMA on HL-60 cells differentiation: mechanism of action. In: Proceedings of the Second International Conference on: Tumor Microenviroment; 2002. p. 221–8.
[24] Eliyahu S, Dovrat S, Albeck M, Segal S, Sredni B. Induction of apoptosis by the immunomodulator AS101 in Ha-Ras transformed cells. In: Proceedings of the Second International Conference on: Tumor Microenviroment Progression; 2002. p. 229–34.
[25] Pluznik DH, Sachs L. The cloning of normal ''mast'' cells in tissue culture. J Cell Physiol 1965;66:319–24.
[26] Taylor WR, Stark GR. Regulation of the G2/M transition by p53. Oncogene 2001;20:1803–15.
[27] Liang YL, Wang LY, Wu H, Ma DZ, Xu Z, Zha XL. PKB phosphorylation and survivin expression are cooperatively regulated by disruption of microfilament cytoskeleton. Mol Cell Biochem 2003;254:257–63.
[28] Deveraux QL, Reed JC. IAP family proteins—suppressors of apoptosis. Genes Dev 1999;13:239–52.
[29] Vanderkerken K, Asosingh K, Braet F, Van R, Van Camp IB. Insulin-like growth factor-1 acts as a chemoattractant factor for 5T2 multiple myeloma cells. Blood 1999;93:235–41.
[30] Tai YT, Podar K, Catley L, Tseng YH, Akiyama M, Shringarpure R, et al. Insulin-like growth factor-1 induces adhesion and migration in human multiple myeloma cells via activation of beta1-integrin and phosphatidylinositol 3′-kinase/AKT signaling. Cancer Res 2003;63:5850–8.
[31] Collins K, Jacks T, Pavletich NP. The cell cycle and cancer. Proc Natl Acad Sci USA 1997;94:2776–8.
[32] Nurse P. Universal control mechanism regulating onset of M-phase. Nature 1990;344:503–8.
[33] Schafer KA. The cell cycle: a review. Vet Pathol 1998;35: 461–78.
[34] Fukuda S, Mantel CR, Pelus LM. Survivin regulates hematopoietic progenitor cell proliferation through p21WAF1/Cip1-dependent and -independent pathways. Blood 2004;103:120–7.
[35] Reed JC. The Survivin saga goes *in vivo*. J Clin Invest 2001;108:965–9.
[36] Altieri DC. Validating survivin as a cancer therapeutic target. Nat Rev Cancer 2003;3:46–54.
[37] O'Connor DS, Grossman D, Plescia J, Li F, Zhang H, Villa A, et al. Regulation of apoptosis at cell division by p34cdc2 phosphorylation of surviving. Proc Natl Acad Sci USA 2000;97:13103–7.
[38] Zaffaroni N, Pennati M, Daidone MG. Survivin as a target for new anticancer interventions. J Cell Mol Med 2005;9:360–72.
[39] Mitsiades N, Mitsiades CS, Poulaki V, Chauhan D, Richardson PG, Hideshima T, et al. Biologic sequelae of nuclear factor-kappaB blockade in multiple myeloma: therapeutic applications. Blood 2002;99:4079–86.
[40] Katayama K, Fujita N, Tsuruo T. Akt/protein kinase B-dependent phosphorylation and inactivation of WEE1Hu promote cell cycle progression at G2/M transition. Mol Cell Biol 2005;25:5725–37.
[41] Tu Y, Gardner A, Lichtenstein A. The phosphatidylinositol 3-kinase/AKT kinase pathway in multiple myeloma plasma cells: roles in cytokine-dependent survival and proliferative responses. Cancer Res 2000;60:6763–70.