Research Article

PK-11195: A POTENTIAL DRUG IN LEUKEMIA TREATMENTG. Mohana Krishna^{1*}, M. Hemanth Kumar², M. Asha Kiran³¹Department of Biochemistry, Andhra University, Visakhapatnam, India²Basavatarakam Indo American cancer hospital and research institute, Hyderabad, India³Department of Biochemistry, Andhra University, Visakhapatnam, India

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DOI: 10.7897/2230-8407.050337**ABSTRACT**

Aims of the present work include 1. Qualifying anti-cancer activity of PK-11195 particularly apoptosis inducing effect on Evi1 expressing 3T3-L1 adipocyte cell lines. 2. Identifying the role of Ca III as an antioxidant in Evi1 expressing 3T3-L1 cell lines. 3. Defining the relation of PK-11195's pro-apoptotic activity to its reported inhibitory action on mitochondrial ATPase. Cell Viability was measured by MTT assay. Apoptosis and caspase activation was measured by caspase 3/7 Glo assay. Western blotting was used to identify protein expression and oxidative stress was measured by Glutathione Reductase assay, Superoxide dismutase assay and Lipid Peroxidation assay. The concluding point that could be made as the results infer is that, by directly inhibiting mitochondrial ATPase PK-11195 induces oxidative stress hence apoptosis in Evi1 expressing 3T3-L1 adipocytes and this sensitivity of 3T3-L1-Evi1 cells is due to the loss of carbonic anhydrase III (Ca III) in those cells.

Keywords: Evi1, mitochondrial ATPase, Oligomycin sensitivity conferring protein, PK-11195 and Ca III

INTRODUCTION

Evi1 was first discovered in 1987 in AKXD-23 recombinant inbred mice that developed myeloid tumors rather than common B and T cell lymphomas¹. Evi1 is a zinc finger transcription factor with two Cys2-His2 zinc finger domains one with 7 zinc fingers at the N-terminal called ZF1 and the other with 3 zinc fingers at the C terminal called ZF2². ZF1 and ZF2 have distinct DNA binding specificities³. There are two distinct repressor domains named Rp⁴ and IR⁵ between ZF1 and ZF2. It contains an acidic domain at C terminus². Evi1 is expressed in adult human kidney, lung, pancreas, brain and ovaries⁶ and is active during embryonic development⁷ and its activity is essential for hematopoietic stem cell self-renewal⁸. HSCs in Evi1^{-/-} embryos are markedly decreased in numbers with defective repopulating capacity⁹. Evi1 is essential for suppression of terminal differentiation of bone marrow progenitor cells⁶. Although Evi1's physiological roles are still the hot topics of research, its pathophysiological roles are better understood in chronic myeloid leukemia (CML), myelodysplastic syndrome (MDS), acute myeloid leukemia (AML) and promyelocytic leukemia. Evi1 is also found to play a role in solid ovarian and colon tumors¹⁰. It has been proposed that Evi1 acts as a survival factor in these tumor cell lines, preventing therapeutic-induced apoptosis and became resistance conferring factor to current treatments¹¹. Peripheral benzodiazepine receptor (PBR) or translocator protein (18 KDa) (TSPO) is a transmembrane protein that is located mainly in the outer mitochondrial membrane and other sub cellular compartments^{12,13}. TSPO is associated with the regulation of cholesterol transport, synthesis of steroid hormones, porphyrin transport, heme synthesis, apoptosis and cell proliferation¹³. A benzodiazepine family member 1-(2-chlorophenyl-N-methylpropyl)-3 isoquinoline carboxamide (PK-11195) is a potential anticancer drug^{14,15}. PK-11195 sensitizes transformed cells to 1) Apoptosis inducing agents like anti-CD95¹⁶, cytokine mda-7/interleukin-24¹⁷,

proapoptotic second messenger ceramide¹⁸, Bcl-2 antagonist HA14.1^{19,20} and anti-CD33 antibody conjugated to gemtuzumab ozogamicin²¹. 2) Apoptosis inducing chemotherapeutic drugs including doxorubicin^{18,20}, daunorubicin²², etoposide^{16,23}, 5-fluorouracil, UV and g-irradiation²³, ifosfamide¹⁶, paclitaxel, docetaxel^{18,20}, colchicine²⁴, arsenicals^{25,26}, lonidamine²⁷ and bortezomib²⁸. Further PK-11195 can also induce apoptosis directly in some cell types¹⁴. Its pro-apoptotic action is both TSPO dependent^{24,29} and independent^{30,31}. Although anti-proliferative and pro-apoptotic actions of PK-11195 are proposed to be mediated through TSPO^{31,32}, ABC transporters³⁰ and phosphodiesterase³³, stronger evidences need to be established. PK-11195 induces caspase dependent apoptosis in CLL cells³⁴. In addition to this PK-11195 has been suggested to induce oxidative stress and this stress generation is upstream to the mitochondrial membrane potential collapse³⁵. Carbonic anhydrase III (CA III) is distinguished from its isozymes by several characteristics, particularly by its low specific activity as a carbon dioxide hydratase and resistance to acetazolamide, which inhibits the isozymes I and II^{36,37}. CA III is abundant in red skeletal muscle, rat liver and in adipocytes³⁸. It is also present in some other tissues and cell types in smaller quantity³⁹. In addition to its carbon dioxide hydratase activity, CA III has a carboxyl esterase activity and tyrosine phosphatase activity⁴⁰. CA III with two of its five cysteine residues undergoes S-glutathiolation⁴¹, which is an early cellular response to oxidative stress⁴². Glutathiolation of CA III is increased during aging and reversibly regulates its tyrosine phosphatase activity⁴³. CA III has two sulfhydryl groups and it is the basis for speculation that it may have a role in scavenging oxygen radicals in skeletal muscle cells^{44,45} and CA III mRNA is induced in muscle of athletes training under hypoxic conditions⁴⁶. Tyrosine phosphatase activity, which is dependent on cellular redox state, suggests that CA III may have a role in intracellular signaling in response to oxidative

stress. A recent study reported that hydrogen peroxide treatment has breached Ev1's anti-apoptotic potential and induced apoptosis in rat fibroblasts expressing Ev1. This apoptosis inducing potency of oxidative stress in these cells is imputed to the transcriptional repression of Ca III by Ev1⁴⁷. A number of apoptosis inducing agents like UV, tumor necrosis factor- α , TGF- β , interferon- α , arsenic toxoid and taxol have been used to study the impact of Ev1 on apoptosis. PK-11195 also induces apoptosis in a number of cases but has not been tested particularly on Ev1 expressing tumors. The objectives of the present work includes 1) Testing anti-cancer effect of PK-11195, particularly apoptosis inducing effect on Ev1 expressing 3T3-L1 cell lines. 2) Finding the role of Ca III as an antioxidant in this phenomenon and 3) Identifying the role of mitochondrial ATPase in this phenomenon.

MATERIALS AND METHOD

Cell Cultures Antibodies and Chemicals

Mouse 3T3-L1 adipocytes endogenously express Ca III. 3T3-L1 cells transfected with Ev1 represented as 3T3-L1-Ev1 was a generous provision from Dr. Krishna Mohan. These stable populations were time to time sub-cultured and incubated at 5 % CO₂. Anti-Ca III (P-17, sc-50715) and anti-Ev1 (E-19, sc-54198), anti-GAPDH (V-18, sc-20357) and rabbit anti-goat IgG-HRP (sc-2768) antibodies and Ca III siRNA (sc-60310) were purchased from Santa Cruz Biotechnology, Inc. Caspase 3/7[®] Glo assay system (G8090) was purchased from Promega. MISSION[®] siRNA Transfection Reagent (S1452) and all the other chemicals were purchased from Sigma-Aldrich Chemicals Pvt. Limited.

Caspase 3 Assay

Cells were incubated in 96-well dishes, relevant indicated cell populations were treated with 70 μ M PK-11195 and apoptosis was determined by Caspase 3 / 7-Glo[®] assay according to the manufacturer's instructions. Luminescence was measured with a luminometer (Centro LB 960).

Western Blotting

Total protein extracts were made from indicated cell populations and performed native PAGE and further this gel was used to transfer band pattern on to PVDF membrane electrophoretically. This PVDF membrane was used to perform western blotting with either of anti-Ca III, anti-Ev1 or anti-gapdh antibodies diluted according to the manufacturer's instructions. Appropriate horseradish peroxidase-conjugated anti-goat IgG was used as secondary antibody at 1/5000 dilutions and detection was performed by enhanced chemi luminescence (Pierce, Rockford, IL, USA, 32209).

MTT Assay

MTT assay was performed on cells grown in 96-well tissue culture plates following treatment with relevant concentration of PK-11195. Cells were treated with 500 μ g/mL MTT in complete medium, 37°C, 5 % CO₂ for 1 h. The medium was removed and replaced with 100 μ l dimethylsulfoxide and absorbance measured at 570 nm using an MRX plate reader (Synergy[™] H1, BioTek). The final absorbance was determined by subtracting the absorbance of treated wells lacking cells. The formazan concentration was determined using the formula:

$$C \text{ (formazan concentration; } \mu\text{M)} = \frac{A \text{ (absorbance)}}{e \text{ (extinction coefficient)} \times l \text{ (path length)}}$$

Sub cellular Fractionation

Mitochondrial fractionation was prepared by differential centrifugation method. 1 ml of trypsinized cell cultures were suspended in Tris – buffer and centrifuged at 1000X g for 10 minutes and the pellet was suspended in 0.25M ice cold sucrose solution and sonicated at 50 % amplitude on ice bucket. This sonicated sample was the centrifuged at 1000x g for 10 minutes at 4°C and the supernatant was taken in the fresh vial. This supernatant was again centrifuged at 12000X g for 10 minutes at 4°C supernatant was discarded and the brown coloured pellet was stored in TAE buffer (50 mM EDTA) pH 8.5 which seems to stabilize the ATPase permitting them to be stored at 4°C for several weeks without significant loss of activity.

Total ATPase Assay

Ascorbate-ammonium molybdate assay was used to measure inorganic phosphate. The ATP hydrolysis assay was conducted in TM buffer (100 mM Tris buffer, 10 mM MgCl₂) at pH 8.5. TM buffer was adjusted appropriately higher than its final pH to neglect the pH drop caused by 10 mM ATP. The linear range of phosphate detected was 1 to 40 nM. The compound is prepared in different concentrations with DMSO and to this 100 μ l of mitochondrial fraction is added and the reaction is carried out 30°C for 15 minutes, at which point it was stopped with 300 μ l of solution containing sodium dodecyl sulfate (SDS), HCl, and ammonium molybdate (0.05 %) and incubated for 10 minutes. Absorbance is measured at 750 nm against blank containing all the components except mitochondrial fraction.

Biochemical Assays

Trypsinized cell cultures were suspended in Tris - buffer and sonicated. These sonicated samples were centrifuged to remove out the membrane debris and used as an enzyme source.

Glutathione Reductase Assay

Glutathione Reductase activity was determined by measuring the decrease in absorbance at 340 nm. The reaction mixture with a total volume of 1 ml contains 100 μ l of 0.5 M potassium phosphate containing 1 mM EDTA (pH 7.8), 810 μ l of distilled water, 50 μ l of enzyme source, 20 μ l of 10 mM NADPH in 50 mM potassium phosphate containing 1 mM EDTA (pH 7.8) and 20 μ l of 10 mM oxidized glutathione (GSSG) in 10 mM potassium phosphate. The reaction mixture was carefully mixed and read at 340 nm in Spectrophotometer (NanoDrop 8000 UV-Vis Spectrophotometer). The activity of glutathione reductase was expressed in terms of μ M NADPH oxidized per mg protein per minute.

Superoxide Dismutase Assay

Superoxide dismutase was assayed. The assay mixture contained 2.4 ml of 50 mM Tris-HCl buffer containing 1 mM EDTA (pH 7.6), 300 μ l of 0.2 mM pyrogallol and 300 μ l enzyme source. The increase in absorbance is measured at 420 nm against blank containing all the components except enzyme and pyrogallol at 10 seconds interval for 3 minutes on Shimadzu UV spectrophotometer. The activity of the enzyme was expressed in μ M of pyrogallol oxidized/min per mg of protein.

Lipid Peroxidation

A break down product of lipid peroxidation, thiobarbituric acid reactive substances (TBARS) was measured. This TBA test involves the reaction of aldehydes with TBA at 100° C under acidic condition to produce a pink colour, which strongly absorbs light at wave length of 535 nm. 100 µl of sonicated sample was added in 900 µl Tris buffer (pH 7.5) then 2 ml of TCA-TBA-HCl reagent was added to this and mixed thoroughly. The solution was incubated for 15 minutes in a boiling water bath. The Flocculent precipitate was removed by centrifuging at 1000X g for 10 minutes. The absorbance of sample was measured at 535 nm against blank that contain no sample. A standard curve was constructed by plotting the amounts of commercially bought product malondialdehyde to the measured absorbance. The TBARS (Thio Barbutiric Acid Reactive Substance) was expressed as µM of malondialdehyde produced/minute.

RESULTS

Evi1 Expressing 3T3-L1 Adipocytes Undergo Apoptosis With PK-11195 Treatment

To determine the effect of PK-11195 treatment on Evi1 expressing fibroblasts 3T3-L1-Evi1 cells were exposed to different concentrations of PK-11195 in DMSO for 12 h. Cell viability monitored by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) assay. Evi1 expression was evident in western blot analysis (Figure 1.A). Histogram shows that cell viability reduced in a PK-11195 dose-dependent manner in both the indicated cell lines and significant reduction in viability is observed in 3T3-L1-Evi1 cells and is most significant at 70 µM PK-11195 (Figure 1.B). This suggests that ectopic Evi1 expression reduces the survival capacity of 3T3-L1 cells on PK-11195 treatment due to suppression of Ca III transcriptionally by Evi1. With the speculation that this cell death could be due to apoptosis, we examined Caspase 3 activity in both the indicated cell lines. The results show here that Caspase 3 catalytic activity is enhanced on PK-11195 treatment for 12 h in both populations but significantly greater in 3T3-L1-Evi1 compared to 3T3-L1 cells. All together this suggests that Evi1 expression in 3T3-L1 adipocytes sensitizes to PK-11195 induced apoptosis.

PK-11195 Treatment Enhances Oxidative Stress in 3T3-L1 Adipocytes Expressing Evi1

Previous studies have shown that PK-11195 induces oxidative stress. Both 3T3-L1 and 3T3-L1-Evi1 cell lines were incubated with 70 µM PK-11195 for 12 h and oxidative stress was assessed by the means of assaying the enzymatic activities of superoxide dismutase (SOD) and glutathione reductase (GR), and measuring the levels of lipid peroxidation. The results show that enzymatic activities of SOD (Figure 4.A) and GR (Figure 4.B), expressed as nM of pyrogallol oxidized/min and nM of NADPH oxidized / min respectively, were reduced in both the indicated cell line extracts but were significantly reduced in 3T3-L1-Evi1 cells on 70 µM PK-11195 treatment for 12 h. Level of lipid peroxidation, expressed as µM malondialdehyde produced / 15 minutes, was also increased in both the indicated cell lines but was most significant among 3T3-L1-Evi1 cell lines on 70 µM PK-11195 treatment for 12 h (Figure 4C). All together this infers that PK-11195 treatment enhances oxidative stress in 3T3-L1 cells expressing Evi1.

Ca III Knockdown Sensitizes 3T3-L1 Adipocytes to Oxidative Stress and Apoptosis Induced By PK-11195

Previous reports suggest that carbonic anhydrase III (Ca III) acts as an antioxidant and protects cell from free radical stress. A recent report states that Ca III is a key role player in hydrogen peroxide induced apoptosis in Evi1 expressing Rat1 fibroblasts. Ca III knockdown experiments were conducted on 3T3-L1-Evi1 with the conjecture of potential involvement of Ca III in induction of oxidative stress and apoptosis on PK-11195 treatment. Western blot analysis was performed to identify effective Dicer-substrate siRNA (DsiRNA) (Figure 3A). These cells were incubated along with the untransfected cells, with PK-11195 for 12 h. Cell viability monitored by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) assay and oxidative stress was assessed by the means of assaying the enzymatic activities of superoxide dismutase (SOD) and glutathione reductase (GR) and measuring the levels of lipid peroxidation. Caspase 3 activity was measured for level of apoptosis. Results show that the knockdown of Ca III is about three fold lower than in untransfected ones (Figure 3A). Significant difference in percent viability is evident between transfected and untransfected cells, but most significant at 70 µM PK-11195 (Figure 3B). We examined Caspase 3 activity in both the indicated cell lines. The results show here that Caspase 3 catalytic activity is enhanced on PK-11195 treatment for 12 h in both populations but significantly greater in Ca III si RNA transfected cells compared to untransfected cells (Figure 3C). Results also show that enzymatic activities of SOD (Figure 5A) and GR (Figure 5B) were reduced both in transfected and untransfected cells, but were significantly reduced in Ca III siRNA transfected cells treated with 70 µM PK-11195 for 12 h. Level of lipid peroxidation was also increased in both the indicated cell lines but was most significant among Ca III siRNA transfected cell lines treated with 70 µM PK-11195 for 12 h (Figure 5C). All together this infers that Ca III knockdown sensitizes 3T3-L1 cells to oxidative stress and apoptosis induced by PK-11195.

PK-11195 Inhibits Mitochondrial ATPase

Reports infer that PK-11195's activities like antimetabolic and suppression of keratinocyte proliferation are owing to its action on OSCP of mitochondrial ATPase. *In vitro* ATPase assay was performed with the speculation of PK-11195's direct action on mitochondrial ATPase. The results obtained narrate the direct action of PK-11195 on mitochondrial ATPase which could be the possible reason behind PK-11195's role in inducing oxidative stress. A dose dependent inhibition was observed where the maximum inhibition was observed at 100 µM of PK-11195.

DISCUSSION

In the present study we show that PK-11195, a peripheral benzodiazepine receptor ligand acts on Evi1 expressing 3T3-L1 cells and induced apoptosis through Caspase 3 activation. Evi1 is a proto-oncogenic zinc finger transcription factor involved in some human neoplasias. It's over expression is observed in acute myeloid leukemia (AML)⁴⁸, myelodysplastic syndromes and chronic myelogenous leukemia⁴⁹⁻⁵² and in some hepatocellular carcinoma⁵³. A full length form of Evi1 is 145 kDa and alternate splicing produces 88 kDa version. In fact its implication in AML is in the form of fusion protein formed by t (3:21) (q26;q22) translocation⁵⁰.

A

B

Figure 1: A) Western blot analysis of whole cell extracts derived from 3T3-L1 and 3T3-L1-Evi1 cells with Evi1 and GAPDH antibodies. The positions of 147 kDa Evi1 and 35 kDa GAPDH proteins are shown. B) Histogram of percentage viability (MTT assay) of untreated cells and cells treated with different concentrations of PK-11195 for 12 h. The columns represent the mean of an experiment performed in triplicate and error bars the standard deviation

Figure 2: Histogram showing relative Caspase 3 activity of the indicated cell line in the absence and presence of 70 μM PK-11195 for 12 h. The columns represent the mean of an experiment performed in triplicate and error bars the standard deviation

A

B

C

Figure 3: A) Western blot analysis using Ca III and GAPDH antibodies, of whole cell protein extracts derived from both untransfected and transfected 3T3-L1 cells with Ca III siRNA. The positions of 27 kDa Ca III and 35 kDa GAPDH proteins are labeled. B) Histogram of percentage viability (MTT assay) of untransfected and transfected 3T3-L1 cells with Ca III siRNA in the presence or absence of 70 µM PK-11195. The columns represent the mean of an experiment performed in triplicate and error bars the standard deviation. C) Histogram of relative Caspase 3 activity of untransfected and transfected 3T3-L1 cells with Ca III siRNA in the presence or absence of 70 µM PK-11195. The columns represent the mean of an experiment performed in triplicate and error bars the standard deviation.

A

B

C

Figure 4: A) Histogram of levels of superoxide dismutase (SOD) in indicated cell line extracts in the presence and absence of 70 μ M PK-11195. The columns represent the mean of an experiment performed in triplicate and error bars the standard deviation. B) Histogram of levels of Glutathione reductase (GR) in indicated cell line extracts in the presence and absence of 70 μ M PK-11195. The columns represent the mean of an experiment performed in triplicate and error bars the standard deviation. C) Histogram of levels of Lipid peroxidation in indicated cell line extracts in the presence and absence of 70 μ M PK-11195. The columns represent the mean of an experiment performed in triplicate and error bars the standard deviation.

Figure 5: A) Histogram of levels of superoxide dismutase (SOD) in indicated cell line extracts in the presence and absence of 70 μ M PK-11195. The columns represent the mean of an experiment performed in triplicate and error bars the standard deviation. B) Histogram of levels of Glutathione reductase (GR) in indicated cell line extracts in the presence and absence of 70 μ M PK-11195. The columns represent the mean of an experiment performed in triplicate and error bars the standard deviation. C) Histogram of levels of Lipid peroxidation in indicated cell line extracts in the presence and absence of 70 μ M PK-11195. The columns represent the mean of an experiment performed in triplicate and error bars the standard deviation.

Figure 6: Measurement of ATPase activity in 3T3-L1 adipocyte mitochondrial fraction at different concentrations of PK-11195. In the histogram the columns represent the mean of an experiment performed in triplicate and error bars the standard deviation

Growth promotion through evasion of apoptosis is a hallmark of tumors. Reports suggest that Evi1 acts as a survival factor and inhibits Rat1 cells from apoptosis and key contributor in the progression of leukemia. Its role as survival factor is supported physiologically as mice require Evi1 to maintain hematopoietic stem cell numbers⁹ and essential in murine development⁷. Evi1 binds to both co-repressors and co-activators and also binds DNA directly and acts as a transcriptional repressor. A well-established example for this role is regulation and induction of GATA2^{9,54}. Microarray analysis in Rat1 cells revealed that Evi1 transcriptionally represses Ca III gene⁴⁷, which protects cells from oxidative stress^{55,56}. Ca III is said to be present in high level in rat liver and decreases during aging so called senescence marker protein⁵⁷ and it is widely accepted that oxygen toxicity may be implicated in aging and in the etiology of a wide variety of pathological conditions like muscular dystrophy and diabetes. Many growth signal-transducing proteins depend on their redox state. Such proteins include growth factor receptors, protein kinases, protein phosphatases, and a number of important transcription factors including NF-kb and AP-1⁵⁸⁻⁶¹. Reports explained catalase as a growth promoting factor⁶² and superoxide dismutase transfected cells exhibited features of cellular senescence⁶³. A strong inference from these is that, oxidative stress regulators could also act as growth regulators by maintaining oxidative stress balance. In the present work Ca III knockdown sensitizes 3T3-L1 cells to apoptosis induced by PK-11195, which strongly infers that PK-11195 could possibly functions through induction of oxidative stress. SOD, GR and lipid peroxidation measurement confirmed that PK-11195 indeed induces oxidative stress. ROS is known to modulate a variety of cellular functions. It has been shown that ROS induces apoptosis in several cell types, including neuronal cells^{64,65}. H₂O₂ and O₂⁻ cause apoptosis through MPT (membrane permeability transition)⁶⁶⁻⁶⁸ and some anticancer drugs induce apoptosis through ROS formation⁶⁹⁻⁷⁶. The present report suggests that PK-11195 can induce caspase dependent apoptosis in Evi1 expressing 3T3-L1 cells. PK-11195 is a high affinity PBR ligand. PBR has a recorded activity as putative therapeutic cell death target^{77,78}. Though PK-11195 has been tested for its ability as an apoptosis inducing agent, the mechanism by which it acts is multifaceted, which include chemo-sensitization to various therapeutic agents and potentiation of TNF toxicity⁷⁹. Another PBR ligand Bz-423 is a pro-apoptotic agent with efficacy against autoimmune diseases in several murine

models^{80,81}. An intimate activity of PBR ligand Bz-423 in suppressing keratinocyte proliferation could be attributed to its ability to bind OSCP (Oligomycine Sensitivity Conferring Protein) and inhibit mitochondrial ATPase⁸². A Report infers that antimitotic activity of high affinity PBR ligand PK-11195 could also be attributed to the action of PBR ligand on mitochondrial ATPase⁸³. Oligomycine sensitivity-conferring protein (OSCP) is a subunit of mitochondrial ATP synthase and is present in the stalk region between the F₀ and F₁ segments. Inhibition of mitochondrial ATPase would lead to increased superoxide, resulting in cell growth arrest and redox regulated apoptosis through membrane permeability transition (MPT)⁸⁴. PK-11195 can potently sensitize to apoptosis induction independent of PBR²⁷. At this juncture it can be hypothesized that PK-11195 may have a direct action on mitochondria. In fact in the present work PK-11195 inhibited mitochondrial ATPase action in mitochondrial sub cellular fraction (Figure 6). Although PK-11195 was tested on AML for its chemosensitizing activity^{30, 21,85}, it was not tested for its individual activity in induction of apoptosis. In fact PK-11195 alone induces apoptosis in chronic lymphoid leukemia (CLL), whereas mechanism is not clear. However no reported properties and interactions of PK-11195 seemed to be involved in this action. It releases cytochrome c and induces caspase-dependent apoptosis in CLL cells¹⁴ and consistently our results showed caspase 3 catalytic activities is increased on PK-11195 treatment.

CONCLUSION

It is inferred based on established reports and results obtained in the present study that PK-11195 causes cell death through induction of oxidative stress. PK-11195 promotes caspase dependent cell death in Evi1 expressing 3T3-L1 adipocytes by increasing cellular oxidative stress due to absence of Ca III dependent antioxidant system. PK-11195 acts through inhibition of mitochondrial ATPase to increase cellular oxidative stress, which goes on its way to induce apoptosis due to lack of Ca III antioxidant system.

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