Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 7 Issue 7 July 2023

Research Article

Orlistat Induced Endoplasmic Reticulum Stress Mediated Apoptosis and Protective Autophagy in PANC-1 Cells: The Key Role of JNK and Mitochondrial Dependent Signalling

Vishal Sharma[1], Raja Ramachandran[2], Samriti Dhawan[3] and Jagdeep Kaur[1]*

[1]*Department of Biotechnology, Panjab University, Chandigarh, India*

[2]*Post Graduate Institute of Medical Education and Research, Chandigarh, India*

[3]*Department of Biotechnology, Goswami Ganesh Dutta Sanatan Dharma College, Chandigarh, India*

***Corresponding Author:** Jagdeep Kaur, Department of Biotechnology, Panjab University, Chandigarh, India.

Received: January 09, 2023

Published: June 27, 2023

Abstract

Orlistat primarily designed to treat obesity is a lipase inhibitor, it is also reported to enhance apoptosis in Human Pancreatic Cancer Cells (PANC-1). Therefore efforts were made in the present investigation to evaluate the effect of orlistat on different parameters playing role in apoptosis and autophagy. Orlistat inhibited the PANC-1 cells growth in a dose-dependent manner with IC_{50} 40 µM after 48 h. Treatment of PANC-1 with 10 µM and 20 µM orlistat for 48 h resulted in autophagy induction. Orlistat treatment of PANC-1 cells caused endoplasmic reticulum stress, as evidenced by increased cytosolic calcium levels, XBP1 splicing, GRP78, and CHOP up-regulation. Orlistat induced ROS generation and translocation of Bax from cytosol to mitochondrion with enhanced cytosolic cytochrome c level. Similarly, a simultaneously enhanced level of cytochrome c was found to be associated with caspase 3 activation and PARP cleavage. These observations suggested that orlistat induced endoplasmic reticulum stress, mitochondrion and ROS mediated cytotoxic action in PANC-1 cells. Further orlistat treatment reveals endoplasmic reticulum stress mediating autophagy through activation of the JNK pathway. To examine whether the autophagy induced by endoplasmic reticulum stress plays a role in cell survival or cell death, autophagy was blocked by 3-Methyladenine. Inhibition of orlistat induced autophagy using 3-Methyladenine results in enhanced apoptosis and suggested protective nature of orlistat induced autophagy in PANC-1. Collectively, all these studies suggested that orlistat had an anti-cancer effect on pancreatic cancer cells. In addition, autophagy played a pro-survival role, suppressing which the orlistat-induced anti-cancer effect would be more significant.

Keywords: Orlistat; Pancreatic Cancer; Autophagy; Endoplasmic Reticulum Stress; Apoptosis

Introduction

Cancer is a global epidemic and the prevalence of total cancer is increasing in developed and developing countries. Pancreatic cancer has been documented for its lethal behaviour and is responsible for 3% of deaths in the US and about 7% of all cancer deaths [1,2]. Recent studies have suggested that macroscopic complete tumor resection and combinational chemotherapeutic options are better preoperative treatments [3-5]. Postoperative adjuvant therapy could further improve the survival rates of pancreatic cancer patients [6]. Furthermore, to augment the survival rate and quality of life new chemotherapeutic molecules with minimal side effects are essentially required. In this aspect orlistat, a fatty acid synthetase (FAS) (EC2.3.1.85) inhibitor is widely under consideration.

Recent studies revealed the role of FAS in development, maintenance and enhancement of the malignant phenotype by shifting the cancer cell metabolism towards the lipids [7-10]. Fatty acid synthetase is associated with fatty acid synthesis from acetyl-CoA, malonyl-CoA and plays a role in cell proliferation [11,12]. Targeting FAS could be a novel approach to reduce malignant phenotypes. In accordance with it many studies revealed the anti-proliferative, apoptotic and antitumor properties of orlistat. Preliminary studies suggested that orlistat inhibited prostate tumor by inhibiting the fatty acid synthetase, while Sokolowaska., *et al.* 2017 suggested that orlistat reduces the proliferation of PANC-1 by inhibiting FAS and induces apoptosis [13-15]. In this regard, pharmacological inhibitors of FAS are increasingly receiving more attention. Therefore the detailed mechanism of induction of apoptosis by orlistat needs to be explored.

Autophagy plays an important role in cell survival and death during certain demanding circumstances including protective role under stress conditions such as starvation, radiation exposure or chemical insults [16]. The process is activated as a safeguard to chemotherapies, and therefore, the regulation of autophagy which sensitizes cell death appears to be therapy for cancer treatment. On the other hand, excessive autophagy stimulates the destruction of malignant cells [17]. Autophagy is a substitute mechanism for cell death in some cancer with natural defects in apoptosis.

In the present investigation, we aim to evaluate the molecular mechanism behind the anticancer and antiproliferative effect of orlistat. Our study revealed that orlistat induced endoplasmic reticulum stress-mediated autophagy in PANC-1 cells. Beside, orlistat induced mitochondrion mediated apoptosis in PANC -1 cancer cells. Furthermore, we also explored the protective nature of autophagy in response to orlistat treatment.

Material and Methods

Cell culture, growth conditions and treatment

PANC-1 cells were procured from National Centre for Cell Science (NCCS) Pune, India. PANC-1 cells were grown in RPMI-1640 medium containing 10% FCS, 100 units penicillin/100 µg streptomycin per ml in CO_2 incubator at 37^{0}C with 95% humidity and 5% CO_2 gas environment. Cells were treated with orlistat dissolved in DMSO while the untreated cultures received only the vehicle (DMSO, <0.2%, v/v). MA (3-Methyladenine) was used as an autophagy inhibitor. The PANC-1 cells were treated with orlistat (10 µM and 20 µM) and MA (3 mM) separately for 48 h alone or in the combination of orlistat 10 µM + MA (3mM) and orlistat 20 µM+ MA (3 mM) [18].

Cell proliferation assay

Cell proliferation was determined using 3-(4,5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. PANC-1 cells (1.0 x 10^4/well) in 96 well culture plates were treated with various concentrations (0-60 µM) of orlistat for 48 h. The MTT formazan crystals formed were dissolved in 200 µl of DMSO and absorbance was measured at 570 nm. The cytotoxicity of orlistat was expressed as the relative viability (% of untreated or control cells).

Acridine orange/ethidium bromide staining

To study the effect of orlistat on autophagy, PANC-1 cells were grown in the presence of 10.0 µM and 20.0 µM of orlistat for 48 h and trypsinized. Further cells were washed two times with phosphate-buffer saline (PBS) and observed under fluorescence microscope (Nikon) after staining with acridine orange/ethidium bromide stain (10 µg/ml).

Quantitative RT-PCR and detection of X-box binding protein 1 (XBP1) splicing

Total RNA was isolated from treated and untreated cells using TRIzol reagent (Sigma, USA). One microgram RNA was used for complementary DNA (cDNA) preparation using Verso cDNA kit (Thermo Scientific, USA). Quantitative RT-PCR analysis was performed in eppendorf real-plex system using the SYBR Green PCR Master mix (Thermo scientific, USA). Quantitative RT-PCR was carried out for CCAAT/enhancer-binding protein homologous protein (CHOP), glucose-regulated protein 78 kDa (GRP78), autophagy related 5 protein (ATG5) genes using gene specific primers [19-21]. For XBP1 splicing XBP1 F (5′-GGTAAGGAACTGGGTCCTT-3′) and XBP R (5′-GAGTTAAGACAGCGCTTGGG-3′) primers were used. An unspliced PCR product of 331 bp was amplified and spliced PCR product of 305 bp was amplified [22]. β-actin served as an internal control. Specificity of PCR products was analyzed using melting curve analysis and delta CT method was used to quantify alteration in gene expression [23].

Flow cytometric analysis

To determine reactive oxygen species (ROS) and intracellular calcium PANC-1 cells (1.0 x 10^6 cells/ml) were treated with orlistat (10 µM and 20 µM) for 48 h. After this cells were washed once in PBS and incubated with Dichloro-dihydro-fluorescein diacetate (DCFH-DA) and Flou 3 AM followed by flow cytomertric analysis as described previously [24]. For flurochrome conjugated antibody staining cells were stained with fluorochrome conjugated antibody anti active caspase 3, anti cytochrome c (Santa Cruz Biotechnology, USA), at 4^0C for one hour. Cells were washed twice with Perm Wash Buffer and subjected flow cytometry analysis. Flow cytometric analysis was performed on a BD FACS Canto II (BD Biosciences, USA) for a maximum cell count of ten thousands and analyzed using BD FACS Diva software.

Western blot analysis

After treatment with orlistat (10 µM and 20 µM) PANC-1 cells were harvested and pellet was washed with cold PBS. Cells were lysed in lysis buffer (20 mM Tris HCl (pH 7.5), 150 mM NaCl, 1% NP-40, 1 mM ethylene glycol-bis (b-aminoethyl ether)-tetraacetic acid, 1 mM EDTA, 50 mM NaF, 1 mM b-glycerophosphate, 2.5 mM sodium pyrophosphate, 1 mM orthovanadate, 1mM protease inhibitor cocktail, 1 mM PMSF) and protein content in the supernatant was determined by protein estimation kit (Bangalore Genie). Equal protein of cell lysate from both treated and untreated was resolved on 10% SDS-PAGE followed by transfer onto a PVDF membrane. Membranes were then probed with anti c-Jun N-terminal kinase (JNK), anti p-JNK, anti p-c-jun, anti c-jun, anti p-p38, anti microtubule-associated protein light chain 3 (LC-3), anti poly ADP ribose polymerase (PARP) antibodies (Sigma USA). Horseradish peroxidise-conjugated secondary antibodies were used to detect immune-reactive bands using 3, 3'-Diaminobenzidine as substrate [25].

Statistical analysis

The data was analyzed statistically using SPSS13.0 and is presented as mean ± standard error of mean (SEM). The results were analyzed using one-way ANOVA to determine the significance of the mean between groups. Values of $P < 0.05$ were considered as significant.

Results

Orlistat inhibited cell proliferation and induced apoptosis.

Dose kinetics of orlistat was determined by treating the PANC-cells with different concentrations of orlistat (0-60 µM) for 48 h. Results revealed IC_{50} of 40 µM (Figure 1 A) and concentration-dependent inhibition of cell growth. Treatment with orlistat at 40 µM significantly reduced the viability of PANC-1 cells after 48 h. Orlistat at concentrations 10 µM and 20 µM (below IC_{50} of 40 µM) were further used for treatment. To reveal the nature of cytotoxicity, DNA laddering experiment was carried out. Orlistat treated cells resulted in the production of characteristic laddering pattern after electrophoresis, while untreated PANC-1 cells revealed the intact band (Figure 1 B).

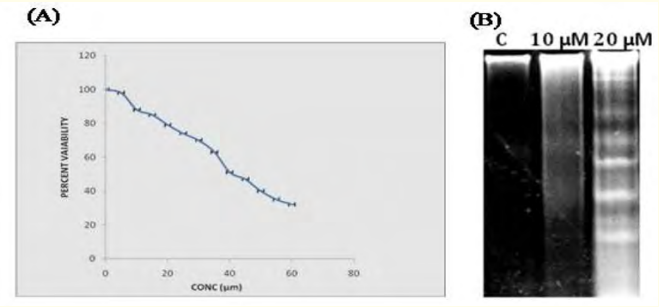

Figure 1: Orlistat inhibited the cell growth and induced apoptosis in PANC-1 cells. (A) PANC-1 cells were treated with different concentrations (0-60 µM) of orlistat for 48 h. MTT assay was used to determine the cytotoxic effect of orlistat. Untreated cells were considered as control (100%). Data are presented as the mean ± S.D. of three similar experiments (B) DNA laddering assay for orlistat treated PANC-1 cells. Control cell revealed intact DNA while orlistat treated cells revealed characteristic laddering pattern.

Orlistat induced autophagy in PANC-1 cells

Orlistat-induced autophagy was demonstrated by acridine orange staining (AOS) of PANC-1 cells, which measures autophagic acidic vesicular organelles (Figure 2 A). Autophagy initiation was further confirmed by using LC3 as a key autophagic marker. Orlistat enhanced the conversion of LC3-I into LC3-II. A significant increase in the expression of LC3-II was observed after 48 h treatment

of orlistat at 10 µM and 20 µM concentration (Figure 2 B) as compared to control. Further, an increase in the expression of ATG5 was observed after 48 h of treatment with 10 µM orlistat, which was further enhanced to 3 folds with 20 µM orlistat in comparison to control (Figure 2 C).

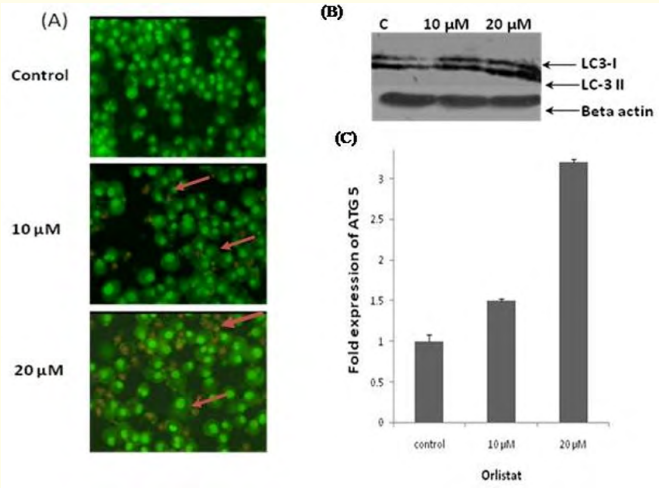

Figure 2: Orlistat induced autophagy in PANC-1 cells. (A) Representative fluorescent microscopy image of PANC-1 cells treated with different concentrations of orlistat 10 µM and 20 µM followed by acridine orange staining. Arrow indicates the presence of autophagic vacuoles (B) Western blots analysis of LC3 conversion after orlistat treatment. Equal protein of total cell lysate after treatment with orlistat was subjected to 10% SDS PAGE followed by western blot hybridization using anti LC3 antibody. (C) Quantitative RT PCR based analysis of relative ATG5 expression. Total RNA was isolated and quantitative RT PCR was carried out with gene-specific primers. The melting curve method was used to detect specificity of amplified gene products. Beta-actin was considered as an internal control. Data are presented as mean ± S.D. of three similar experiments. Untreated cells were considered as control.

Orlistat induced endoplasmic reticulum stress and enhanced cytosolic calcium

Since orlistat has been shown to induce endoplasmic reticulum stress (ERS) mediated pathways, therefore we investigated the effect of orlistat on the calcium homeostasis [26]. Flow cytometric analysis revealed that treatment of PANC–1 with orlistat lead to nearly 2.5 fold increase in intracellular cytosolic calcium level as compared to control (Figure 3 A, B). Further, orlistat treatment resulted in the significant endoplasmic reticulum stress generation which was confirmed by the XBP-1 splicing and up-regulation of GRP78, CHOP genes expression. Treatment with the 20 µM orlistat resulted in XBP1 splicing and 5 folds up-regulation of CHOP expression as compared to the control (Figure 3 C, D). While there were 6 fold up-regulation in the expression of the GRB78 gene (Figure 3 E) as compared to control.

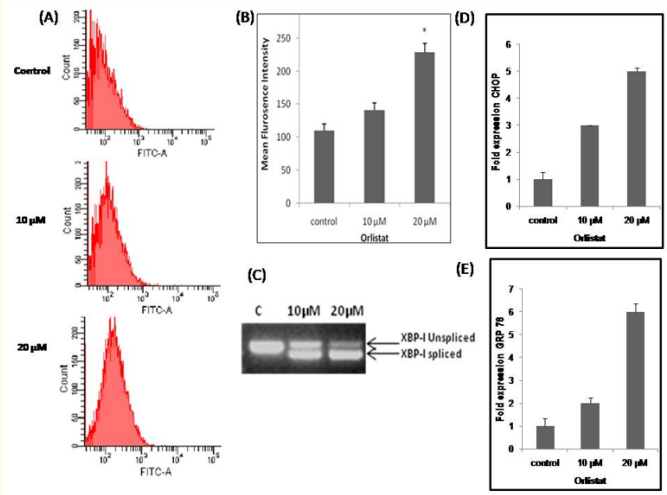

Figure 3: Orlistat induced the endoplasmic reticulum stress in PANC-1 cells. (A) Representative FACS analysis histogram of PANC-1 cells treated with orlistat. Cells were treated with different concentrations of orlistat (10 µM, 20 µM) and subjected to flou3 AM staining followed by flow cytometric analysis. Ten thousand events were counted per tube. (B) Bar graphs showing change in the total cytosolic calcium level expressed as mean fluorescence intensity. (C) Orlistat treatment-induced XBP1 splicing and (D, E) up-regulation of CHOP and GRP78 gene expression. Total RNA was isolated after orlistat treatment and RT PCR was carried out using gene-specific primers. Melting curve analysis was used to determine the specificity of amplified PCR products. Beta-actin considered as an internal control. Data are presented as mean ± S.D. of three similar experiments. Untreated cells were considered as control. P value < 0.05 was taken as significant.

Orlistat induced significant ROS generation

It has been documented by the various researches that ROS has an essential key role in the autophagic and apoptosis induction pathways [27,28]. To gain a better understanding of orlistat mediated apoptosis induction, we accessed the level of ROS. A significant change in the mean fluorescent intensity was observed after treatment with the orlistat. Treatment with the 20 µM orlistat depicted the nearly 2.5 fold increase in ROS mean fluorescence intensity as compared to control (Figure 4).

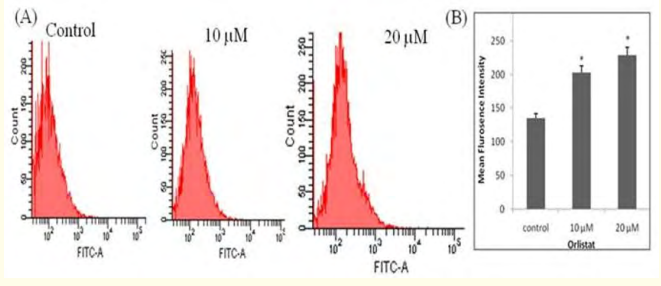

Figure 4: Orlistat induced ROS in PANC-1 cells. (A) Orlistat treatment induced ROS in PANC-1 cells. Representative FACS analysis histograms of DCFH-DA stained orlistat treated (10 µM, 20 µM) PANC-1 cells. Ten thousand events were counted per tube (B) Bar graph represents the change in the level of ROS expressed as mean fluorescence intensity. Data are presented as mean ± S.D. of three similar experiments. Untreated cells were considered as control. P value < 0.05 was taken as significant.

Orlistat induced mitochondrion and caspase 3 mediated cell death

The Bax previously have been documented to play role in the apoptosis induction by mitochondrion mediated pathways [29]. Therefore, we evaluated the PANC-1 cells after orlistat treatment for distribution of Bax in cytosolic and mitochondrial fractions. Results suggested that treatment of PANC-1 cell with orlistat shown translocation of Bax from cytosol to the mitochondrion fraction (Figure 5 A) resulting in enhanced cytochrome c release (Figure 5 B, E). It has been found that PANC -1 cells upon treatment with orlistat lead to enhanced expression of downstream effector caspase 3 (Figure 5 D, F). The PARP is down steam effector of the caspase. Treatment of PANC-1 with the orlistat resulted in the cleavage of the PARP and appearance of the 86 kDa band (Figure 5 C). Collectively these results suggested that orlistat induced the mitochondrion and caspase mediated apoptosis induction.

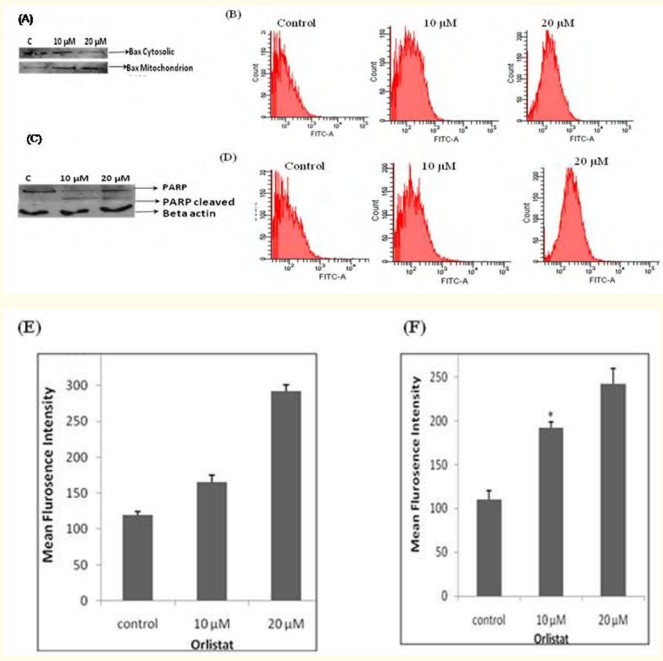

Figure 5: Orlistat induced mitochondrion and caspase mediated apoptosis in PANC-1 cells. (A) Distribution of Bax in cytosolic and mitochondrion fractions after orlistat (10 µM, 20 µM) treatment. Sub cellular fractionation was performed to obtain the fractions for cytosol and mitochondrion followed by western blot analysis using anti Bax antibody. (B) Representative FACS analysis histogram of the enhanced release of cytosolic cytochrome c level after orlistat treatment. PANC-1 cells were treated with orlistat followed by staining with fluorochrome-labeled anti-cytochrome c antibody. Ten thousand events were counted per tube (C) PARP cleavage after orlistat treatment of PANC-1 cells. PANC-1 cells treated with orlistat (10 µM, 20 µM) shown cleaved band as compared to control. Total cell lysate was resolved on 10% SDS-PAGE for immunoblot analysis with anti PARP antibody. β-actin was taken as an internal control (D) FACS analysis histogram PANC-1 cell were treated with orlistat (10 µM, 20 µM) followed by staining fluorochrome labelled anti active caspase 3 antibody. Ten thousand events were counted per tube (E) Bar graph represents the change in cytoplasmic cytochrome c expressed as mean fluorescence intensity. (F) Bar graph represents the change in expression of active caspase 3 expression expressed as mean in fluorescent intensity. Data are presented as mean ± S.D. of three similar experiments. Untreated cells were considered as control. P < .05 was considered as significant.

P-JNK/p-c-Jun participated in orlistat induced ERS mediated protective autophagy in PANC-1 cells

We next sought to determine how autophagy and ERS contributed to cell death. Previous studies have established the role of JNK/c-jun pathway in ERS mediated autophagy [30]. Therefore the role of orlistat induced ERS mediated autophagy through activation of JNK pathway was investigated. The western blotting revealed enhanced expression of p-JNK and p-c-jun, p-p38, (Figure 6 A), while no significant differences in the levels of total JNK was found. Further attempts were made to investigate whether autophagy induction is the result of cell survival or cell death signals. PANC-1 cells were exposed to orlistat treatment in the presence and absence of MA, an autophagy inhibitor. The drugs combination (orlistat + MA) reduced the cell viability in comparison to orlistat alone and promoted apoptosis (Figure 6 D). These results suggested that autophagy inhibition enhanced the apoptotic effect of orlistat. The results were further supported by the enhanced expression of active caspase 3 under similar conditions (Figure 6 B, C). These studies pointed towards the protective autophagy in pancreatic cancer cells after orlistat treatment.

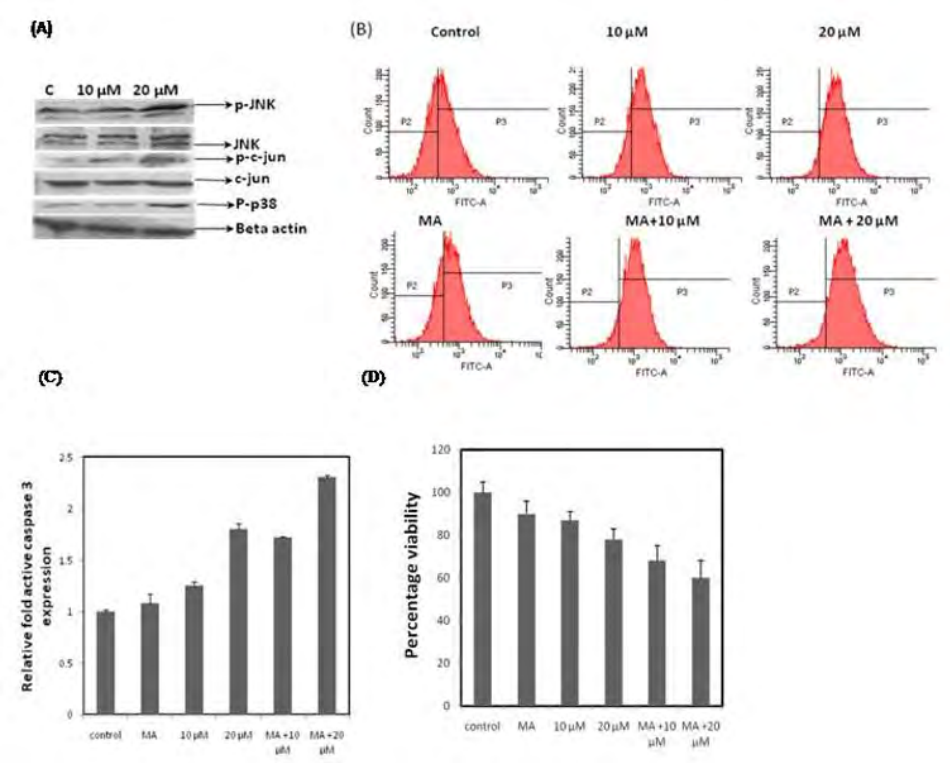

Figure 6: Orlistat induced ERS mediated protective autophagy in PANC-1 cells. (A) The change in expression pattern of JNK, p-JNK, c-jun, p-c-jun, p-p38 after orlistat treatment (10 µM, 20 µM). PANC-1 cells were treated with different concentrations of orlistat for 48 h and total cell lysates were resolved on 10% SDS-PAGE for immunoblot analysis of JNK, p-JNK p-c-jun, c-jun, p-p38. Beta actin was used as an internal control. (B) Representative FACS analysis histogram of active caspase 3 in PANC-1 cells treated with different concentration orlistat and MA (in presence and absence of MA and combination of orlistat + MA) as described in material and method section. Level of active caspase 3 was determined using the flow cytometry after staining with flurochrome conjugated anti active caspase 3 antibody. Ten thousand events were counted per tube (C) Bar graph represents change in active caspase 3 expression expressed as mean fluorescence intensity (D) Percentage viability of cells after orlistat treatment (in presence and absence of MA and combination of orlistat + MA). Data are presented as mean ± S.D. of three similar experiments. Inhibition of autophagy with MA resulted in enhanced expression of active caspase 3 decreased viability in cells treated with orlistat and MA combination as compared to orlistat alone treated or untreated cells.

Discussion

Pancreatic cancer is projected to become the most deadliest cancer by 2040 and the overall 5-year survival rate is currently in less than 7% cases [31]. Reprogramming of metabolic pathways is characteristic of cancer cells. Recent studies have established a significant correlation between the actively growing cancer cells and reprogrammed lipid metabolism of various cancers [11]. Lipid synthesis plays a significant role in metastasis and also drug resistance in cancer. Orlistat has been previously documented for its role in the induction of anti-proliferative effect and apoptotic activity in PANC-1 cells. However, to develop it as an anticancer molecule, its complete molecular mechanism needs to be evaluated. In the present study, we studied the mechanism of action of orlistat on PANC-1 cells in detail.

Orlistat treatment resulted in DNA laddering and suggested the apoptotic effect of orlistat in accord with earlier studies [15]. Bax is a crucial protein known for its role in apoptosis induction [29]. Further, the orlistat treatment resulted in the activation of the mitochondrial apoptosis pathway as revealed by the increase in cleavage of caspase-3, PARP, translocation of Bax from the cytoplasm to the mitochondrion and enhanced cytochrome C release from mitochondria. Taken together, these results documented that orlistat induced the cytotoxicity in PANC-1 cells through promoting apoptosis, which was mediated by the mitochondrial apoptosis pathway.

Orlistat has been documented for its potential to induce the ROS in prostate cancer cells and shown to sensitize the cells [32,33]. Our studies are in agreement with these studies and suggested the role of ROS in orlistat mediated cytotoxicity in PANC-1 cells.

Cumulating evidence suggested the interrelation of ERS and ROS with redox signalling. The endoplasmic reticulum is regularly involved in folding and maturation of proteins to maintain the homeostatic conditions in the cells [34]. Consistent activation of ERS can lead to apoptosis and autophagy [27,35]. Upregulation of ERS marker and enhanced cytosolic calcium in orlistat treated PANC-1 cells confirmed the ERS.

Published literature established the role of ERS in the induction of autophagy and apoptosis in cancer cells [36,37]. To explore the nature and biological mechanism behind orlistat mediated toxicity, cells were subjected to acridine orange staining and LC3 western blot analysis. Induction of autophagy seems a primary event during orlistat treatment. Orlistat treatment contributed to apoptosis and ERS-mediated autophagy in PANC-1cells. However, in the literature studies, the interaction between autophagy and apoptosis is still not fully understood. Earlier literature predicted the JNK/p38 pathway role in the ERS mediated autophagy [38]. Shen., *et al.* (2017) suggested the JNK pathway role in 18β-glycyrrhetinic acid associated ERS mediated autophagy [39]. Further animal model studies have shown that JNK activation is crucial for the induction of autophagy in Bax/Bak double-knockout mice [40]. The results from the present study demonstrated orlistat induced autophagy in PANC-1cells (as evidenced by an increase of the expression levels of LC3-I to LC3-II, Atg5, and acridine orange studies), and activation of JNK/p38 pathway that might have contributed to ERS mediated autophagy in PANC-1 cells. There are lots of contradictions existing in the literature about the role of autophagy in cancer for instance; some well-known conventional agents could positively inhibit tumor growth when used in combination with chemotherapeutic agents or radiation by regulating the autophagy process [41,42]. In consistent with apoptosis, autophagy-mediated suppression or promotion of cancer depends on tumor types or microenvironment [43]. The relationship between autophagy and apoptosis is still contradictory. Genistein has been also shown to enhance the antitumor effect of 5-fluorouracil by inducing apoptosis and autophagy [44]. In contrast, Talukdar., *et al.* (2018) revealed the protective nature of autophagy by MDA-9/Syntenin which led to anoikis resistance of glioblastoma stem cells [45]. Xu., *et al*. (2017) reported that Deguelin treatment resulted in protective autophagy in human pancreatic cancer cells, and apoptosis-induced by Deguelin could be enhanced by suppressing autophagy [46]. In agreement with Xu., *et al.* 2017 in this case also, blocking orlistat induced autophagy dramatically enhanced cell apoptosis in pancreatic cancer cells. However, why FAS inhibition activated autophagy and ERS in PANC-1 cells is still speculative:

Conclusion

The present study revealed that orlistat induced apoptosis in the PANC-1 cells by ERS, ROS and mitochondrion mediated pathways. Moreover, orlistat induced ERS led to the occurrence of

pro-survival autophagy mediated by JNK/c-jun activation. Further inhibition of autophagy may result in significant anti-cancer potential of orlistat. However, these prospective anti-cancer effects will be more valuable to be further investigated in animal models and clinical trials.

Disclosure

The authors have no competing interests.

Acknowledgement

This study was supported by Council of Scientific and Industrial Research, New Delhi, India.

Bibliography

1. Rahib L., *et al*. "Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States". *Cancer Research* 74.11 (2014): 2913-2921.

2. Matrisian LM and Berlin JD. "The past, present, and future of pancreatic cancer clinical trials". *American Society of Clinical Oncology - Educational Book* 35 (2016): e205-215.

3. Souchek JJ., *et al*. "Combination treatment with orlistat-containing nanoparticles and taxanes is synergistic and enhances microtubule stability in taxane-resistant prostate cancer cells". *Molecular Cancer Therapeutics* 16.9 (2017): 1819-1830.

4. Chiou YS., *et al*. "Prevention of breast cancer by natural phytochemicals: focusing on molecular targets and combinational strategy". *Molecular Nutrition and Food Research* 62.23 (2018): e1800392.

5. Qu CY., *et al*. "Engineering of lipid prodrug-based, hyaluronic acid-decorated nanostructured lipid carriers platform for 5-fluorouracil and cisplatin combination gastric cancer therapy". *International Journal of Nanomedicine* 10 (2015): 3911-3920.

6. Long J., *et al*. "Cancer statistics: current diagnosis and treatment of pancreatic cancer in Shanghai, China". *Cancer Letter* 346.2 (2014): 273.

7. Ballinger A and Peikin SR. "Orlistat its current status as an anti-obesity drug". *European Journal of Pharmacology* 440.2-3 (2002): 109-117.

8. Swierczynski J., *et al*. "Role of abnormal lipid metabolism in development, progression, diagnosis and therapy of pancreatic cancer". *World Journal of Gastroenterology* 20.9 (2014): 2279-2303.

9. Kuhajda FPL. "Fatty-acid synthase and human cancer: new perspectives on its role in tumor biology". *Nutrition* 16.3 (2002): 202-208.

10. Zaidi N., *et al*. "Lipogenesis and lipolysis: the pathways exploited by the cancer cells to acquire fatty acids". *Progress in Lipid Research* 52.4 (2013): 585-589.

11. Menendez JA and Lupu R. "Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis". *Nature Reviews Cancer* 7.10 (2007): 763-777.

12. Kuhajda FP. "Fatty acid synthase and cancer: new application of an old pathway". *Cancer Research* 66.12 (2006): 5977-5980.

13. Kridel SJ., *et al*. "Orlistat is a novel inhibitor of fatty acid synthase with antitumor activity". *Cancer Research* 64.6 (2004): 2070-2075.

14. Sadowski MC., *et al*. "The fatty acid synthase inhibitor triclosan: repurposing an anti-microbial agent for targeting prostate cancer". *Oncotarget* 5.19 (2014): 9362-9381.

15. Sokolowska E., *et al*. "Orlistat reduces proliferation and enhances apoptosis in human pancreatic cancer cells (PANC-1)". *Anticancer Research* 37.11 (2017): 6321-6327.

16. Codogno P., *et al*. "Autophagy and signaling: their role in cell survival and cell death". *Cell Death and Differentiation* 2 (2015): 1509-1518.

17. Yu L., *et al*. "The selectivity of autophagy and its role in cell death and survival". *Autophagy* 4.5 (2008): 567-573.

18. Liu D., *et al*. "Inhibition of autophagy by 3-MA potentiates cisplatin-induced apoptosis in esophageal squamous cell carcinoma cells". *Medical Oncology* 28.1 (2011): 105-101.

19. Wang FM., *et al*. "Resveratrol triggers the pro-apoptotic endoplasmic reticulum stress response and represses pro-survival XBP1 signaling in human multiple myeloma cells". *Experimental Hematology* 39.10 (2011): 999-1006.

20. Lin WC., *et al*. "Endoplasmic reticulum stress stimulates p53 expression through NF-κB activation". *PLoS One* 7.7 (2012): e39120.

21. Alirezaei M., *et al*. "Elevated ATG5 expression in autoimmune demyelination and multiple sclerosis". *Autophagy* 5.2 (2009): 152-158.

22. Zhu S., *et al*. "Endoplasmic reticulum stress mediates aristolochic acid I-induced apoptosis in human renal proximal tubular epithelial cells". *Toxicology In Vitro* 26.5 (2012): 663-671.

23. Livak KJ and Schmittgen TD. "Analysis of relative gene expression data using real-time quantitative PCR and the 2 (-Delta Delta C (T)) Method". *Methods* 25.4 (2001): 402-408.

24. Kumar A., *et al*. "A novel parthenin analog exhibits anti-cancer activity: activation of apoptotic signaling events through robust NO formation in human leukemia HL-60 cells". *Chemico-Biological Interactions* 193.3 (2011): 204-215.

25. Watanabe Y., *et al*. "Interferon-gamma induces reactive oxygen species and endoplasmic reticulum stress at the hepatic apoptosis". *Journal of Cellular Biochemistry* 89.2 (2003): 244-253.

26. Little JL., *et al*. "Inhibition of fatty acid synthase induces endoplasmic reticulum stress in tumor cells". *Cancer Research* 67.3 (2007): 1262-1269.

27. Yen YP., *et al*. "Arsenic induces apoptosis in myoblasts through a reactive oxygen species-induced endoplasmic reticulum stress and mitochondrial dysfunction pathway". *Archives of Toxicology* 86.6 (2012): 923-933.

28. Liu ZW., *et al*. "Protein kinase RNA-like endoplasmic reticulum kinase (PERK) signaling pathway plays a major role in reactive oxygen species (ROS)-mediated endoplasmic reticulum stress-induced apoptosis in diabetic cardiomyopathy". *Cardiovascular Diabetology* 12.1 (2013): 1-6.

29. Sharma V., *et al*. "Low-pH-induced apoptosis: role of endoplasmic reticulum stress-induced calcium permeability and mitochondria-dependent signaling". *Cell Stress Chaperones* 20.3 (2015): 431-440.

30. Raciti M., *et al*. "JNK2 is activated during ER stress and promotes cell survival". *Cell Death and Disease* 3.11 (2012): e429.

31. Rawla P., *et al*. "Epidemiology of Pancreatic Cancer: Global Trends, Etiology and Risk Factors". *World Journal of Oncology* 10.1 (2019): 10-27.

32. Wen X., *et al*. "Deconvoluting the role of reactive oxygen species and autophagy in human diseases". *Free Radical Biology and Medicine* 65 (2013): 402-410.

33. Wright C., *et al*. "Anti-Tumorigenic Potential of a Novel Orlistat-AICAR Combination in Prostate Cancer Cells". *Journal of Cellular Biochemistry* 118.11 (2017): 3834-3845.

34. Zhao L and Ackerman SL. "Endoplasmic reticulum stress in health and disease". *Current Opinion in Cell Biology* 18.4 (2006): 444-452.

35. Ogata M., *et al*. "Autophagy is activated for cell survival after endoplasmic reticulum stress". *Molecular and Cellular Biology* 26.24 (2006): 9220-9231.

36. Ding WX., *et al*. "Differential effects of endoplasmic reticulum stress-induced autophagy on cell survival". *Journal of Biological Chemistry* 282.7 (2007): 4702-4710.

37. Cheng Y and Yang JM. "Survival and death of endoplasmic-reticulum-stressed cells: Role of autophagy". *World Journal of Biological Chemistry* 10 (2011): 226-231.

38. Zheng Y., *et al*. "Pinocembrin induces ER stress mediated apoptosis and suppresses autophagy in melanoma cells". *Cancer Letter* 431 (2018): 31-42.

39. Shen S., *et al*. "Blocking autophagy enhances the apoptotic effect of 18β-glycyrrhetinic acid on human sarcoma cells via endoplasmic reticulum stress and JNK activation". *Cell Death and Disease* 8.9 (2017): e3055.

40. Shimizu S., *et al*. "Involvement of JNK in the regulation of autophagic cell death". *Oncogene* 29.14 (2010): 2070-2082.

41. Sui X., *et al*. "Autophagy and chemotherapy resistance: a promising therapeutic target for cancer treatment". *Cell Death and Disease* 14.10 (2013): e838.

42. Sui X., *et al*. "p38 and JNK MAPK pathways control the balance of apoptosis and autophagy in response to chemotherapeutic agents". *Cancer Letter* 344.2 (2014): 174-179.

43. Xu Y., *et al*. "Active autophagy in the tumor microenvironment: A novel mechanism for cancer metastasis". *Oncology Letter* 5.2 (2013): 411-416.

44. Suzuki R., *et al*. "Genistein potentiates the antitumor effect of 5-Fluorouracil by inducing apoptosis and autophagy in human pancreatic cancer cells". *Anticancer Research* 34.9 (2014): 4685-4692.

45. Talukdar S., *et al*. "MDA-9/Syntenin regulates protective autophagy in anoikis-resistant glioma stem cells". *Proceedings of the National Academy of Sciences of the United States of America* 115.22 (2018): 5768-5773.

46. Xu XD., *et al*. "Inhibition of Autophagy by Deguelin Sensitizes Pancreatic Cancer Cells to Doxorubicin". *International Journal of Molecular Sciences* 18.2 (2017): 370.