Targeting Bcl-2 Protein to Enhance Chemosensitivity of Hormone Refractory Prostate Cancer Cell Line, DU-145 by a Synergistic Combination of Docetaxel and Gossypol

Hormona Refrakter Prostat Kanseri Hücre Dizisi DU-145'in Kemoterapi Duyarlılığını Artırmak İçin Dosetaksel ve Gossipolün Sinerjik Bileşimi

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ABSTRACT Objective: Docetaxel has become the standard of care for hormone-refractory prostate cancer (HRPC), however drug resistance and toxicity are still challenging in daily cancer practice. The anti-apoptotic pathway centered around the Bcl-2 protein might be one of the responsible pathways. Adding a second drug to docetaxel treatment is one of the most common approaches to solve this problem. Gossypol was reported to have potent anticancer activities in prostate cancer. In this study, we searched for the possible synergistic cytotoxic/apoptotic effects of this combination treatment in hormone- and drug resistant prostate cancer cell line, DU-145 via inhibition of Bcl-2 protein. **Material and Methods:** XTT cell viability assay was used to assess cytotoxicity of the drugs alone and in combination. For verifying apoptosis, Cell Death Detection Elisa Plus Kit and Caspase-Glo 3/7 assay were used. Western Blot analysis for Bcl-2 protein was carried out. **Results:** A novel drug combination of docetaxel and gossypol resulted in a significant synergistic cytotoxic activity and apoptosis as compared to any single agent alone, in a dose- and time dependent manner, and also significantly reduced Bcl-2 protein levels in DU-145, in doses that can be used clinically. **Conclusion:** Adding gossypol to docetaxel as a combination treatment in HRPC patients might be a solution for taxane resistant patients. Inhibition of Bcl-2 might be one of the underlying routes of activity for this combination.

Key Words: Docetaxel; gossypol; prostatic neoplasms

ÖZET Amaç: Dosetaksel, hormona dirençli prostat kanserinin (HRPC) tedavisinde standart tedavi haline gelmiş durumdadır ancak ilaç direnci ve toksisite sorunları uygulamada hala mevcudiyetini devam ettirmektedir. Apoptoz karşıtı olan Bcl-2 proteini prostat kanserinde ilaç direncinden sorumlu yollardan biri olabilir. Bu sorunu çözmek için en çok kullanılan yöntem dosetaksele ikinci bir ilaç eklemektir. Gossipolün prostat kanserinde kuvvetli anti-kanser etki gösterdiği bildirilmiştir. Bu çalışmada, DU-145 adlı hormona ve ilaca dirençli prostat kanser hücresi dizisinde, dosetaksel/gossipol kombinasyonunun Bcl-2 protein inhibisyonu yoluyla gösterebileceği olası sinerjik sitotoksik/apoptotik etkiyi araştırdık. **Gereç ve Yöntemler:** İlaçların tek başına ve kombinasyon halinde hücreler üzerinde yaptığı toksik etki, hücre canlılığını belirleyen XTT yöntemiyle araştırıldı. Apoptozu doğrulamak için Cell Death Detection Elisa Plus Kit ve Caspase-Glo 3/7 yöntemleri kullanıldı. Western Blot yöntemiyle Bcl-2 analizi yapıldı. **Bulgular:** Yeni bir ilaç kombinasyonu olan dosetaksel ve gossipol, ilaçların tek başına yaptığına oranla belirgin sinerjik sitotoksik aktivite gösterdi ve ilaçların her birinden daha fazla apoptoza neden oldu. Bu etki dozla ve zamanla artan bir etkiydi ve klinikte kullanılabilecek dozlarda DU-145 hücre dizisinde Bcl-2 proteinini azalttı. **Sonuç:** HRPC hastalarında dosetaksele gossipol eklenerek yapılacak kombinasyon tedavisi taksan direnci için bir çözüm olabilir. Kombinasyonun etki mekanizmalarından biri de Bcl-2 inhibisyonu olabilir.

Anahtar Kelimeler: Dosetaksel; gossipol; prostat tümörleri

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Altered balance between antiapoptotic and proapoptotic molecules may cause tumor development and/or progression.[1] The antiapoptotic Bcl-2 family proteins play a pivotal role in the regulation of apoptosis in many types of human cancers, including prostate cancer.[2] Some of the members of this family such as Bcl-2 and Bcl-X_L are antiapoptotic while others such as Bad, Bax or Bid are pro-apoptotic.[2-4] Overexpression of Bcl-2 protein seems to enable the prostate cancer cells to survive in an androgen-deprived environment and protect them to undergo apoptosis. Excess antiapoptotic protein, Bcl-2 in prostate cancer cells, will result in resistance to treatment.[5] Thus, Bcl-2 might be a very useful target for the treatment of hormone-refractory prostate cancer (HRPC).

The majority of metastatic prostate cancer patients respond to androgen deprivation treatment initially, however most will become castrate resistant within approximately two-years of time.[6] Docetaxel, the most effective chemotherapeutic agent for HRPC, shows its activity through stabilizing microtubules during cell division. In addition to this cytostatic activity, it also regulates cell signaling and expression of certain genes. Docetaxel treatment increases Bcl-2 phosphorylation, down regulates Bcl-X_L protein levels, induces p53 and antiangiogenic factors and thus results in apoptosis.[6-8] Although docetaxel chemotherapy has become the first-line standard of care for HRPC based on the results of two large randomized trials, prostate specific antigen (PSA) responses rarely exceed 50% and median survival is less than 20 months. Thus chemotherapy in this clinical state remains a subject of active clinical investigation.[9,10] There are also some problems encountered during docetaxel treatment including serious side effects in most of the patients.[11] Investigators are now focusing on how to enhance the cytostatic and cytotoxic effects of docetaxel by combining it with novel anticancer agents for the treatment of prostate cancer.

Gossypol is a natural polyphenolic compound extracted from cotton plant (*Gossypium species*) and the tropical tree, *Thespesia populnea.* Recently, gossypol was reported to have potent anticancer activities in many types of malignancies, including prostate cancer. The Antitumoral and Biochemical Effects of Gossypol on human cell lines were investigated.[12] Gossypol was shown to be a potent inhibitor of Bcl-2/Bcl-X_L. However, the exact mechanisms responsible for inhibition of cell growth and stimulation of apoptosis have not been elucidated yet.[13-15]

In this study, we aimed to investigate the possible synergistic cytotoxic/apoptotic effects of a novel combination, docetaxel and gossypol in hormone- and drug refractory prostate cancer cells, DU-145. We also searched for the possible enhanced effect in down regulation of Bcl-2 protein since both docetaxel and gossypol are known to be effective on the inhibition of Bcl-2 in HRPC.

MATERIAL AND METHODS

CELL LINE AND REAGENTS

Human DU-145 prostate cancer cells were obtained from ICLC (Genova, Italy). The cells were grown as monolayers in adherent cell lines and were routinely cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% L-glutamine, 1% penicillin-streptomycin in 75 cm^2 polystyrene flasks (Corning Life Sciences, UK) and maintained at 37°C in a humidified atmosphere with 5% CO_2. Growth and morphology were monitored and cells were passaged when they had reached 90% confluence. Cell culture supplies were obtained from Biological Industries (Kibbutz Beit Haemek, Israel). Docetaxel and gossypol (≥98% purity) were obtained from Sigma Chemical Co (USA). The stock solution of docetaxel (10 mM) and gossypol (10 mM) were prepared in dimethyl sulfoxide (DMSO). The final dilutions were made immediately before use, and new stock solutions were made for each experiment. The DMSO concentration in the assay did not exceed 0.1% and was not cytotoxic to the tumor cells. All other chemicals, unless mentioned, were purchased from Sigma. Anti- *Bcl-2* antibody and Beta-Actin antibody were obtained from Amersham (Munich, Germany).

VIABILITY ASSAY

After verifying cell viability using trypan blue dye exclusion test by Cellometer automatic cell counter

(Nexcelom Inc.,USA.), cells were seeded at approximately $1x10^4$/ well in a final volume of 200 µl in 96-well flat-bottom microtiter plates with or without various concentrations of drugs. Plates were incubated at 37°C in a 5% CO_2 incubator for the indicated time periods. At the end of incubation, 100 µl of XTT (Roche Applied Science, Mannheim, Germany*)* was added to each well, and plates were incubated at 37°C for another 4 h. Absorbance was measured at 450 nm against a reference wavelength at 650 nm using a microplate reader (DTX 880 Multimode Reader, Beckman Coulter, Fullerton, CA, USA). The mean of triplicate experiments for each dose was used to calculate the IC_{50} and the combination index (CI) values.

EVALUATION OF APOPTOSIS

Measurement of DNA Fragmentation by ELISA Assay

Apoptosis was evaluated by enzyme-linked immunosorbent assay (ELISA) using Cell Death Detection ELISA Plus Kit (Roche Applied Science, Mannheim, Germany) according to the instruction manual. The relative amounts of mono- and oligonucleosomes generated from the apoptotic cells were quantified using monoclonal antibodies directed against DNA and histones by ELISA. Briefly, cytoplasmic fraction of the untreated control and docetaxel and/or gossypol treated cells were transferred onto a streptavidin-coated plate and incubated for 2 h at room temperature with a mixture of peroxidase conjugated anti-DNA and biotin labeled antihistone. The plate was washed throughly, incubated with 2.29-Azino-di-[3-ethylbenzthiazolinesulfonate] diammonium salt (ABTS), then absorbance was measured at 405 nm with a reference wavelength at 490 nm (DTX 880 Multimode Reader, Beckman Coulter, Fullerton, CA, USA). The kit contains %100 apoptotic cell lysate as an intra-assay detection control. Thus, relative apoptosis was calculated based on %100 apoptotic cell lysate absorbance value in our experiments.

Measurement of Caspase 3/7 Enzyme Activitiy

The Caspase-Glo 3/7 assay (Promega, Madison, WI, USA) was used to measure caspase 3/7 enzyme activity according to the manufacturer's instructions. DU-145 cells at a concentration of 10^4cells/well were plated in a 96-well plate in 100 µl culture medium in the absence or presence of increasing concentrations of docetaxel or gossypol and the combination of both. 100 µl of Caspase-Glo 3/7 reagent was added to each well and incubated at room temperature for one more hour. Finally, the luminescence of each sample was measured with luminometer (DTX 880 Multimode Reader, Beckman Coulter, Fullerton, CA, USA).

WESTERN BLOT ANALYSIS

DU-145 cells were grown for 72 h in the absence or presence of docetaxel (1nM) or gossypol (5 µM) and, in combination of both at 37°C. To prepare cell lysates for Western immunoblot analysis, cell pellets were washed with phosphote buffered saline (PBS), and lysed in buffer containing 20 mM Tris (tris(hydroxymethyl)aminomethane)-HCl (pH8.0), 137 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM Na3VO4, 25 mM -glycerophosphate and protease inhibitor cocktail 1 (Sigma, St Louis, MO, USA). After centrifugation at 14,000*g* for 15 min at 4°C, protein concentrations were quantitated by Bradford method (Bio-Rad Laboratories, Hercules, CA). Equal amounts of protein were separated on an SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to polyvinylidene difluoride immobilon-P membranes (Bio-Rad Laboratories, Hercules, CA). The membranes were blocked with 5% nonfat drymilk prepared in Tris-buffered saline containing 0.1% Tween 20 at room temperature for 1 h, and then incubated with primary monoclonal Bcl-2 and beta-actin antibodies (Amersham, Munich, Germany) at room temperature for 1 h. Dilutions of primary antibodies were prepared according to the manufacturer's instructions. Following several washes in tris buffered saline and Tween 20 (TBST), membranes were incubated with appropriate secondary antibodies (1:2000) at room temperature for 1 h. The protein bands recognized by the antibodies were visualized by the ECL Western blotting detection system according to the protocol provided by the manufacturer (Amersham, Munich, Germany) using Kodak Gel Logic 1500 Imaging System.

STATISTICAL ANALYSIS

The data were analyzed using GraphPad PRISM software (version 5) (San Diego, CA, USA). All experiments were set up in triplicate and the results were expressed as the mean ± standard deviation (SD). The paired Student's t- test was used to compare the differences between paired samples. Differences were considered significant at p value below 0,05. Median dose effect analysis was used to assess the interaction between the agents. The combination index (CI) values were calculated by using Biosoft CalcuSyn program (Ferguson, MO, USA) and CI was used to express synergism (CI<1), additive effect (CI= 1), or antagonism (CI >1).[16]

RESULTS

CELL GROWTH INHIBITORY EFFECTS OF DOCETAXEL AND GOSSYPOL IN DU-145 CELLS

To evaluate the effects of docetaxel and gossypol on the viability of human prostate cancer cells, DU-145 cells were exposed to increasing concentrations of docetaxel (from 0.01-to 100 nM) and gossypol (from 3- to 20 μM) for 24, 48 and 72 h, and XTT cell viability assay was performed. Both docetaxel and gossypol decreased cell viability in a time- and dose dependent manner in DU-145 cells (data not shown). As shown in Figure 1, there were 8-, 15-, and 43% decreases in cell viability of DU-145 cells exposed to 0.1-, 1-, and 10 nM of docetaxel, respectively, when compared to untreated controls at 72 h (Figure 1). Highest cytotoxicity was observed at 72 h and IC_{50} value of docetaxel in DU-145 cells calculated from cell viability plots was 15 nM.

In addition to this, our data showed that incubation of DU-145 cells with increasing concentrations of gossypol resulted in significant inhibition of viability. In parallel with those results, there were 5-, 29- and 61% decreases in cell viability of DU-145 cells in response to 3-, 7.5 and 15 μM of gossypol, respectively, as compared to untreated controls at 72 h (Figure 2). IC_{50} value of gossypol was 10 μM for DU-145 cells.

COMBINATION OF DOCETAXEL AND GOSSYPOL CAUSED INHIBITION OF CELL GROWTH SYNERGISTICALLY IN DU-145 CELLS

In order to examine the effects of docetaxel and gossypol combination on cell viability, DU-145 cells were treated with increasing concentrations of both drugs for 72 h. Combination of various concentrations of docetaxel and gossypol showed significant growth suppression in human prostate cancer cell line DU-145 as compared to any agent alone as shown in Table 1.

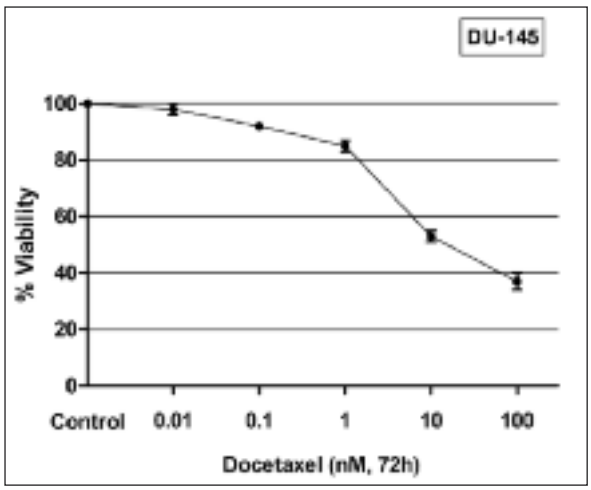

FIGURE 1: Effects of docetaxel in DU-145 cells. Viability was assessed by XTT viability assay following 72 h culture. The data represent the mean of three different experiments (p<0.05). The error bars represent the standard deviations, and when not seen, they are smaller than the thickness of the lines on the graphs.

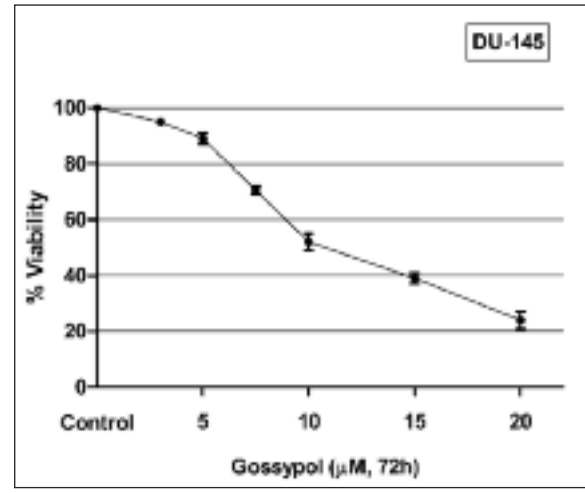

FIGURE 2: Effects of gossypol on DU-145 cells. Viability was assessed by XTT viability assay following 72 h culture. The data represent the mean of three different experiments (p<0.05).The error bars represent the standard deviations, and when not seen, they are smaller than the thickness of the lines on the graphs.

TABLE 1: Combination index (CI) values of DOC (docetaxel)/gossypol in DU-145 cells. Combination index (CI) values were calculated from the XTT viability assays, according to CalcuSyn® software. The CI was used to express synergism (CI < 1), additive effect (CI= 1), or antagonism (CI > 1), where CI<0.5 represents strong synergism.

Concentration of DrugsCI	Value	Interpretation
DOC (0.1 nM) + Gossypol (5 μM)	0.294	Strong Synergism
DOC (1 nM) + Gossypol (5 μM)	0.276	Strong Synergism

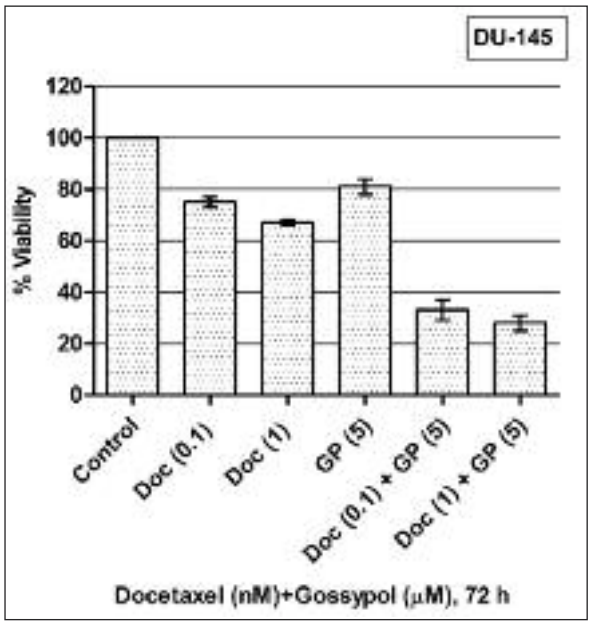

FIGURE 3: Synergistic effects of docetaxel and gossypol on viability of DU-145 cells. Viability was determined by the XTT viability test in a 72-hour culture. The results are expressed as the mean of three different experiments ($p<0.05$). The error bars represent the standard deviations, and when not seen, they are smaller than the thickness of the lines on the graphs.

The XTT viability data indicated that 0.1 nM docetaxel and 5 μM gossypol resulted in 25%- and 19% decreases in viability of DU-145 cells, respectively, while the combination treatment, at the same concentrations, resulted in 70% decrease in viability, as compared to untreated controls (Figure 3).

The CI values were calculated to be 0.294 or 0.276 in DU-145 cells that were exposed to 0.1- or 1 nM docetaxel and 5 μM gossypol, respectively; indicating that the combination of docetaxel and gossypol at these concentrations showed a strong synergism (Table 1).

EFFECTS OF THE SEQUENTIAL TREATMENT

The previous findings demonstrated that treatment of tumor cells with docetaxel and gossypol resulted in significant synergistic activity at 72 h. We examined the effect of sequential treatment of DU-145 cells with either docetaxel or gossypol and subsequent treatment with the second agent. Pretreatment of tumor cells with docetaxel for 36 h and then wash and treat with gossypol for an additional 36 h resulted in synergistic cytotoxicity in DU-145 cells. In addition, pretreatment of tumor cells with gossypol for 36 h and then wash and treat with docetaxel for an additional 36 h resulted in synergistic cytotoxicity in DU-145 cells (data not shown). Therefore, synergistic effect of the combination treatment was obtained, no matter which agent applied first.

A SIGNIFICANT INCREASE IN DNA FRAGMENTATION WAS DETECTED IN RESPONSE TO COMBINATION TREATMENT OF DOCETAXEL AND GOSSYPOL AS COMPARED TO ANY AGENT ALONE IN DU-145 CELLS

To examine the induction of apoptosis in response to docetaxel or gossypol and combination of both in DU-145 cells, we incubated the cells in the presence of agents alone or in combination of both for 72 hours and then quantified the levels of mono-oligo nucleosome fragments by Cell Death Detection Kit (Roche Applied Science, Mannheim, Germany). The results of DNA fragmentation analyses clearly showed that combination of both agents induced apoptosis in a synergistic manner as compared to any agent alone.

As shown in Figure 4, there were 1.6- or 1.5-fold increases in DNA fragmentation in 0.1 nM docetaxel or 5 μM gossypol exposed DU-145 cells, respectively compared to untreated controls, while the combination of both resulted in 9-fold increase in DNA fragmentation. On the other hand, in 1 nM docetaxel or 5 μM gossypol *or* combination of both exposed DU-145 cells, there were 1.9- or 1.5- and 10.3 fold increases in DNA fragmentation, respectively compared to untreated controls. Data in Figure 4 show that combination of two drugs induced apoptosis significantly through DNA fragmentation as compared to any agent alone.

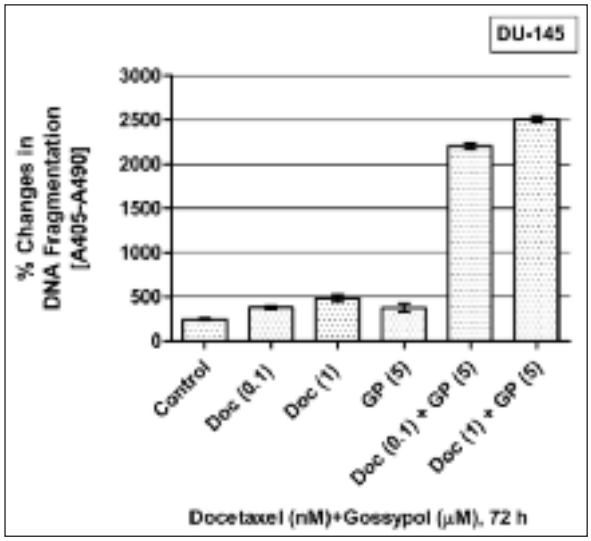

FIGURE 4: Apoptotic effects of docetaxel and gossypol alone or in combination in DU-145 cells through DNA fragmentation analyses. The results are the means of two independent experiments ($p<0.05$). The error bars represent the standard deviations, and when not seen, they are smaller than the thickness of the lines on the graphs.

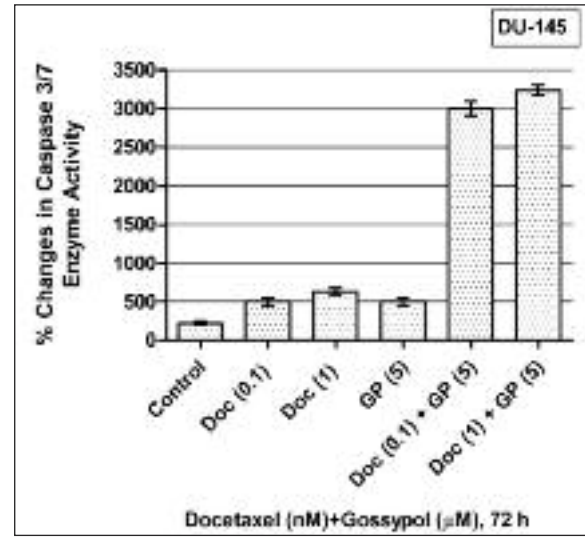

FIGURE 5: Apoptotic effects of docetaxel and gossypol alone or in combination in DU-145 cells through caspase 3/7 enzyme activity analyses. The results are the means of two independent experiments ($p< 0.05$). The error bars represent the standard deviations, and when not seen, they are smaller than the thickness of the lines on the graphs.

CASPASE 3/7 ENZYME ACTIVITY HAS BEEN INCREASED IN RESPONSE TO COMBINATION OF DOCETAXEL AND GOSSYPOL MORE THAN ANY AGENT ALONE IN DU-145 CELLS

Caspases might be termed as hangmen of apoptosis. The activation of many forms of caspases is seen in many cells undergoing apoptosis. To evaluate whether caspases play role in docetaxel and gossypol-induced apoptosis of DU-145 cells, we measured the levels of caspase 3/7 using Caspase-Glo 3/7 Assay.

The results revealed that there was a dose dependent increase in caspase 3/7 enzyme activity in docetaxel or gossypol treated DU-145 cells. DU-145 cells exposed to 1 nM docetaxel and 5 μM gossypol showed 2.7- or 2.3 fold increases in caspase 3/7 enzyme activity, respectively compared to untreated controls, while their combination resulted in 14.2 fold increase in caspase 3/7 enzyme activity (Figure 5).

DOCETAXEL AND GOSSYPOL INDUCED APOPTOSIS IS PARTLY CONTROLLED BY THE ENHANCED DOWN-REGULATION OF ANTIAPOPTOTIC BCL-2 PROTEIN IN DU-145 CELLS

Since Bcl-2 is one of the target proteins for both docetaxel and gossypol treatment in HRPC, we performed Western Blot analyses to examine whether the combination of two resulted in a synergistic down-regulation of antiapoptotic Bcl-2 protein in DU-145 cells. We have clearly shown that there was a significant decrease in protein levels of Bcl-2 in DU-145 cells exposed to 1 nM docetaxel and 5 μM gossypol compared to any agent alone (Figure 6). These data indicated that combination of docetaxel and gossypol induced apoptosis in DU-145 cells through the down regulation of antiapoptotic Bcl-2 protein.

DISCUSSION

Data presented here provide the evidence that treatment of hormone- and drug resistant prostate cancer cell line, DU-145 with a novel combination, docetaxel and gossypol, results in a significant synergistic cytotoxic activity and apoptosis compared to any single agent alone. This effect was observed in a dose- and time dependent manner. It was also shown that apoptosis was induced in prostate carcinoma cells with significant cytotoxicity, no matter which agent was applied first. Additionally, we demonstrated that there were drug concentration dependent increases in DNA fragmentation and caspase 3/7 enzyme activity in prostate carcinoma cells exposed to docetaxel or gossypol alone, but the combination of two resulted in synergistic activity.

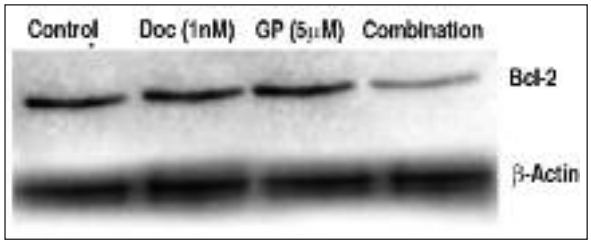

FIGURE 6: Demonstration of protein levels of antiapoptotic Bcl-2 in DU-145 cells exposed to docetaxel and gossypol alone and in combination of both. Protein levels of Bcl-2 were determined by Western blotting and beta actin protein levels were measured as an internal positive control.

Impaired balance between pro- and anti-apoptotic pathways is accepted as a major contributing factor in the process of carcinogenesis. Among those proteins related to apoptosis, up-regulation of Bcl-2 is frequently associated with poor prognosis, drug resistance and short of survival in several types of cancers, including prostate cancer.[16-22]

Since Bcl-2 overexpression, of which observed in a high percentage of HRPC patients, impedes apoptosis and has a crucial role in transition from androgen-dependent to androgen independent tumor growth, many studies are going on in order to find an optimal solution for Bcl-2 overexpression.[23] This protein contributes to resistance to docetaxel. Possible enhancement of the efficacy of docetaxel in HRPC by combining the agent with oblimersen, a fully phosphorothioated antisense oligonucleotide that selectively down-regulates Bcl-2 protein expression is under investigation in a recent trial. Previous phase I and II trials of oblimersen as a chemosensitizing agent before docetaxel therapy have yielded promising results.[24] Thus, Bcl-2 might be a potential surrogate marker and a target molecule for taxane-based treatments in HRPC.

Based on this knowledge, it is clear that new treatment approaches are needed for docetaxel sensitization in HRPC. Similar to the effects of docetaxel on Bcl-2 protein in cancer cells, gossypol, a natural product from cottonseed, has recently been identified as a potent small molecule inhibitor of both Bcl-2 and Bcl-xL.[25] There are studies demonstrating the fact that gossypol increases the sensitivity of prostate cancer cells to conventional cytotoxic treatment, without causing any significant toxicity.[26] Our team has been working with gossypol since 2007 in our research laboratory, and previously we have also shown that drug resistance and metabolism gene expression patterns have been changed with combination treatment of docetaxel and gossypol in hormone- and drug refractory PC-3 prostate cancer cells. In that study, we have demonstrated that five important genes (BRCA1, CCND1, ERBB2, RB1 and TPMT) associated with either drug resistance or metabolism were downregulated by ≥ 3 fold by the combination treatment by PCR- Array method. However, the level of alteration in Bcl-2 family genes could not reach to statistically significance. Since, PC-3 and DU-145 are different cell lines by means of both phenotypical and genotypical properties, the same combination treatment might work in different routes of cellular resistance.[27] We also believe that working with other prostate cancer cells like DU-145, in our study, will better let us to elucidate cause and effect relationships of this new drug combination for HRPC.

In the present study, we have identified that the combination treatment of docetaxel and gossypol significantly reduced Bcl-2 protein levels in hormone- and drug refractory prostate cancer cells, DU-145, at doses that can be used clinically. Since existence of Bcl-2 is associated with drug resistance and aggressive nature of HRPC, inhibition of this antiapoptotic protein may lead to overcome resistance to apoptosis in HRPC cells. Thus, adding gossypol to docetaxel as a combination treatment in HRPC patients might be one of the solutions for patients who develop failure or resistance to taxane therapy.

CONCLUSION

In conclusion, inhibition of Bcl-2 protein by the combination treatment of docetaxel and gossypol might be one of the critical routes underlying the strong synergistic cytotoxic and apoptotic effect of both in hormone-and drug refractory prostate cancer cell line, DU-145. From a clinical point of view, simultaneous application of these drugs with strong synergy in inducing apoptosis in DU-145 cells might allow reduction in docetaxel doses and diminish docetaxel related adverse effects while maintaining the therapeutic effect in patients with HRPC.

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