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ARAŞTIRMA / RESEARCH

Investigation of apoptotic effects of D-pantothenic acid on PC-3 prostate cancer cells

D-pantotenik asidin PC-3 prostat kanseri hücreleri üzerindeki apoptotik etkilerinin incelenmesi

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Abstract

Purpose: The anti-inflammatory and antioxidant properties of D-pantothenic acid have been demonstrated and the effects of dexpentanol on inflammatory pathways and apopototic pathways that trigger cell death are of interest. Apoptotic pathways are important in resistance to chemotherapeutics in cancer diseases and in cancer development. Therefore, we planned how treatment of PC-3 human prostate cancer cells with dexpanthenol will affect the levels and activities of apoptotic and inflammation mediators. For this purpose, human prostate cancer cell culture was performed.

Materials and Methods: The human prostate cancer cells were treated with dexpentanaol then protein levels and activities of inflammatory and apoptotic pathway mediators such as gadd153, AIF, grp78, bax and bcl-2 in the cells were analyzed by ELISA.

Results: The results of our study showed that, D-pantothenic acid did not statisticaly decreased the leves of bax, bcl-2 and grp78 protein expression in PC-3 prostate cancer cells. The effect of D-pantothenic acid on gadd153 and AIF proteins in PC-3 cells was increased but this increased level did not statisticaly significant.

Conclusion: Recent studies have demonstrated the potential benefits of anti-inflammatory drugs. Our study showed that D-pantothenic acid had no significant effect on the growth of PC-3 cells and has no significant effect on intracellular apoptotic pathways.

Keywords:. D-pantothenic acid, prostate cancer, dexpanthenol, apoptosis

Öz

Amaç: D-pantotenik asidin anti-enflamatuar ve antioksidan özellikleri gösterilmiştir ve dekspentanolün hücre ölümünü tetikleyen enflamatuar yolaklar ve apopototik yollakar üzerindeki etkileri, merak uyandırmaktadır. Apoptotik yolaklar, kanser hastalıklarında ve kanser gelişiminde kemoterapötiklere karşı dirençlilik açısından önemlidir. Bu nedenle, PC-3 insan prostat kanseri hücrelerinin dekspantenol ile tedavisinde apoptotik ve inflamasyon mediyatörlerinin seviyelerini ve aktivitelerini nasıl etkileyeceğini planladık. Bu amaçla, insan prostat kanseri hücre kültürü yapıldı.

Gereç ve Yöntem: İnsan prostat kanseri hücreleri, dekspentanaol ile muamele edildi ve hücrelerde gadd153, AIF, grp78, bax ve bcl-2 gibi enflamatuar ve apoptotik yolaklardaki aracıların protein seviyeleri ve aktiviteleri ELISA ile analiz edildi.

Bulgular: Çalışmamızın sonuçları, D-pantotenik asidin PC-3 prostat kanseri hücrelerinde bax, bcl-2 ve grp78 protein ekspresyonu düzeylerini istatistiksel olarak azaltmadığını gösterdi. PC-3 hücrelerinde D-pantotenik asidin üzerindeki etkisi, gadd153 ve AIF proteinleri arttırıldığı, ancak bu artış seviyesi istatistiksel olarak anlamlı değildi.

Sonuç: Son çalışmalar, anti-enflamatuar ilaçların potansiyel faydalarını göstermiştir. Çalışmamız D-pantotenik asidin PC-3 hücrelerinin büyümesi ve hücre içi apoptotik yolaklar üzerinde anlamlı bir etkisinin olmadığını göstermiştir.

Anahtar kelimeler: D-pantotenik asid, prostat kanseri, inflamasyon ve apoptozis

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INTRODUCTION

Prostate cancer (PC) is the most common disease in Europe and the United States as a first and in the world and Turkey is the second[1]. Prostate cancer risk factors include aging, familial predisposition, genetics, race, diet, hormonal and environmental factors[2]. 30% of patients diagnosed with prostate cancer have advanced disease. Also, 25% of patients are included in the advanced disease group during their follow-up[3]. Screening studies with prostate specific antigen (PSA) have shown that the chance of catching the disease at an early stage increases and mortality decreases. Cure can be achieved with radical treatment in patients at this stage[4]. Hormonal therapy is the first form of treatment for patients who cannot cure with local radical therapy[5]. With hormone ablation therapy in these patients progression survival time is between 12 and 33 months[6]. Many patients respond symptomatically and biochemically to treatment. However, treatment does not prolong survival and ultimately enters an androgen-independent stage in all patients. These patients respond to some degree of secondary hormonal interventions, However, despite hormonal treatments, these patients progress inevitably and become hormone-resistant[7]. At this point, various chemotherapy protocols and experimental approaches are on the agenda to increase survival and provide an effective palliative treatment[8]. In addition, chemotherapy is not only used in hormone resistant in the treatment of prostate cancer. Chemotherapy can be used in high-risk localized prostate cancer in order to reduce recurrence after or before radiotherapy and radical prostatectomy, and before adding some mutations induced by hormone ablation therapy that may decrease the chemotherapy response[9].

In the process of apoptosis embryogenesis, in removing the unwanted tissues and reshaping the tissue; The subsequent years are programmed cell death, which functions in achieving development, homoestasis, and eliminating damaged, transformed, infectious tissue and healthy tissue that has expired during the entire life cycle. Research in recent years on the induction or blockade of apolitic signal molecules has brought a new perspective to the control of cancer development. The Bcl-2 family and caspases are the main mediators in the apoptotic pathway. It activates caspase-3, with activation of intrinsic pathway caspase-8 and 10, and the formation of death-inducing signal complex, with activation of extrinsic pathway caspase-9 and apoptosome complex. In this last step, caspase-3 causes typical DNA fragmentation. The bcl-2 family has both apoptotic and anti apopitotic members. According to the balance between Bcl-2 proteins, proapopitotic or antiapopitotic signals affect mitochondria. If apopitotic signals are predominant, the cytochrome c leaves the mitochondria to form the apoptosome complex. Triggered apoptosis causes organ failure, while inhibition of apoptosis causes hyperplasia and cancer[10,11]. Apoptosis defects are important in developmental, autoimmune and neurodegenerative diseases and cancer development. There are studies showing that anti-apoptotic genes are responsible for resistance to chemotherapeutics in cancers. Anti-apoptotic effect bcl-2 protein family in many cancer types levels were determined to be induced[12,13,14].

In cancer, apoptosis has an important role in resistance to chemotherapeutics and cancer development. Recent studies have revealed the potential benefits of anti-inflammatory drugs. The dexpentanal that we use in our study has anti-inflammatory activity. We predict that in apoptosis known as dexpentanol cell death, bax and bcl-2, which act as mediators, and inos, cox-2, cpla2 and nfkb, which affect inflammation, can affect protein levels and activities.. In our study, it was aimed to investigate the effects of D-pantothenic acid on protein levels and activities of gadd153, AIF, grp78, bax and bcl-2 in human prostate cancer cells.

MATERIALS AND METHODS

Cell culture

Human prostate cancer cells PC-3 (ATCC® CRL-1435) were grown on 10% Fetal Bovine Serum (FBS) (Hyclone), 1% L-Glutamine (Hyclone), 1% Penicillinstreptomycin (Hyclone), Dulbecco's modification of Eagle's medium (DMEM) (GIBCO). Cells were grown as single layer in $1x10^5$ number in 24 standard cell culture plates. Incubated at 37°C and 5% CO_2 oven. These cells were treated with 3mM pentehtenic acid and homogenized for protein analysis by ELISA method.

Tissue homogenization

3ml RIPA (Radio-Immunoprecipitation Assay) buffer, 30µl PMSF (phenylmetanesulfonylfluoride), 30µl sodium vanadate, 30µl protease inhibitor were added to the cells and homogenates were obtained by dissecting the tissues on ice. The homogenates are

centrifuged at 10,000 RPM for 10 minutes and the supernatants separated from the top, the precipitates (pellets) are discarded.

Protein quantification

Protein quantification of homogenized Cells was done by Bradford method. Using bovine serum albumin (1µg / ml), a standard is prepared at concentrations of 1, 2, 3, 5, 7, 8, 10 (µg / ml) and 10 µl is taken from each sample and completed to 100 µl with distilled water. After adding 1ml of Bradford solution on the standard and samples and mixing with vortex, the absorbance amounts at 595 nanometer wavelength were measured manually in the spectrophotometer. Protein amount determination was made in µg / µl according to the standard curve drawn in Prism program. Protein quantification was performed for the standardization of ELISA experiments.

ELISA (Enzyme Linked Immunosorbent Assay) Test

Bax, bcl-2, gadd153, grp78, and AIF protein levels were examined by ELISA test. Experiment protocols of ELISA kits vary for each kit.

As studies on such commercially available human cell line (Human prostate cancer cells PC-3 (ATCC® CRL-1435) do not require an ethics committee approval, we did not obtain any ethical approval to conduct the study.

Statistical analysis

Student's t-test was applied to the variables to reveal the difference between the groups. The values of $p < 0.05$ were considered statistically significant in the interpretation of the results. The results are presented as mean ± standard deviation (SD) or min-max.

RESULTS

Bcl-2 expression in treatment with 30lg / mL D-pantothenic acid after 48 hr incubation did not significantly decreased in prostate cancer compared to control ($p > 0.05$).

AIF expression in treatment with 30 lg / mL D-pantothenic acid after 48 hours incubation did not significantly increase the amount of AIF protein, which is the anti apoptotic protein in prostate cancer compared to control. ($p > 0.05$).

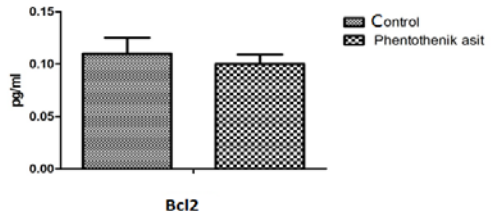

Figure 1. Effect of D-pantothenic acid treatment on Bcl-2 expression

Bax expression in treatment with 30lg / mL D-pantothenic acid after 48 hours of incubation the expression of anti-apoptotic protein in PC-3 prostate cancer compared to control did not decreased significantly ($p > 0.05$).

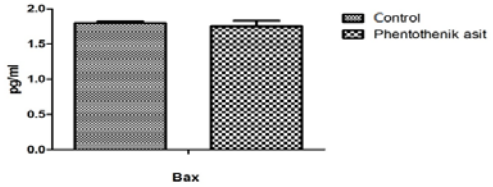

Figure 2. Effect of D-pantothenic acid treatment on Bax expression

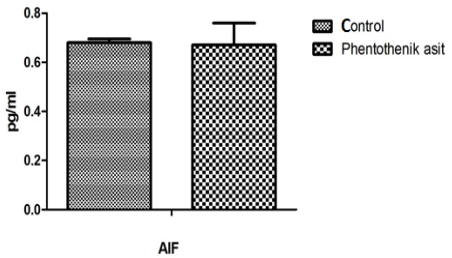

Figure 3. Effect of D-pantothenic acid treatment on AIF expression

GADD153 expression in treatment with 30lg / mL D-pantothenic acid after 48 hours of incubation did not significantly increase the expression of anti-apoptotic protein in prostate cancer compared to control ($p > 0.05$).

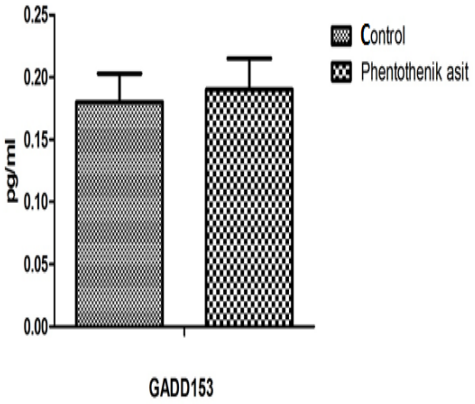

Figure 4. Effect of D-pantothenic acid treatment on GADD153 expression

GRP78 expression in treatment with 30lg / mL D-pantothenic acid after 48 hours of incubation did not significantly decreased the expression of anti-apoptotic protein, GRP78, in prostate cancer compared to control (p >0.05).

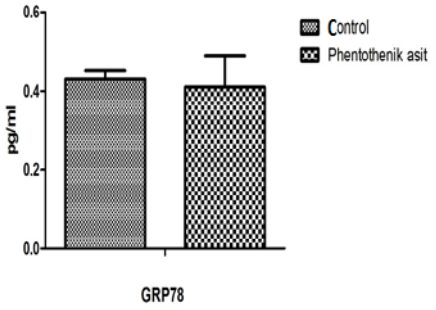

Figure 5. Effect of D-pantothenic acid treatment on GRP78 expression

Table 1. Effects of D-pantothenic acid on expression of Bcl-2, Wee1, GADD153 AIF and GRP78 proteins

	Control	Pantothenic acid
Bcl-2	0.11±0.02 pg/ml	0,10±0.08 pg/ml
bax	0.67±0.023 pg/ml	0.60±0.025 pg/ml
AIF	0.68±0.015 pg/ml	0.71±0.09 pg/ml
gadd153	0.18±0.023 pg/ml	0.19±0.025 pg/ml
grp78	0.43±0.02 pg/ml	0.41±0.08 pg/ml

Results are presented as Mean±SE, statistical analysis: Student t-test, *Control p<0.05

DISCUSSION

Prostate cancer is a type of cancer that causes the death of a large number of men every year. In screening in Turkey PK is the second most common type of cancer after lung cancer incidence was calculated as 24,33/100000. Lifelong predictable risk of PC is 16.72%, while death risk is 2.57% [15,16].

Despite recent advances, prostate cancer continues to be the main cause of cancer-related mortality and morbidity in men. Androgen suppressive treatments are first applied to prostate cancer patients, but most patients also develop a resistant form of prostate cancer that does not respond to androgen suppressive treatments. This cancer without any curative treatment is known as castration resistant prostate cancer (CRPC). With the development of new strategies for prostate cancer treatments, it is desirable to increase survival outcomes in patients who are in this lethal form[17,18].

In the recent years, because of increasing in awareness of usage of complementary and alternative medicines in health management and believing that "natural" chemicals are always safe. Vitaminler, vücut büyümesi, gelişimi ve metabolik fonksiyonlar da dahil olmak üzere normal fizyoloji için esasen ihtiyaç duyulan yapısal ve işlevsel olarak ilgisiz doğal kimyasallar grubudur. Dengeli, sağlıklı bir gıda, vücutta ihtiyaç duyulan tüm vitaminleri destekler. D-pantotenik asidin eksikliklerinin veya aşırı ancak tolere edilebilir kaynaklarının PC gelişimi veya ilerlemesi üzerinde etkileri olup olmadığı bu çalışmada araştırmaya tabi tutulmuştur[19,20,21].

The Bcl-2 family and caspases are the main mediators in the apoptotic pathway. It activates caspase-3, with activation of intrinsic pathway caspase-8 and 10 and the formation of death-inducing signal complex, with activation of extrinsic pathway caspase-9 and formation of apoptosome complex. In this last step, caspase-3 causes typical DNA fragmentation. The Bcl-2 family has both apoptotic and anti apoptotic members. According to the balance between Bcl-2 proteins, proapopitotic or antiapopitotic signals affect mitochondria. If apoptotic signals are dominant, cytochrome c leaves the mitochondria to form the apoptosome complex. while inhibition of apoptosis causes hyperplasia and cancer, Triggered apoptosis causes organ failure. Apoptosis defects are important in developmental, autoimmune, neurodegenerative diseases and cancer development19. In our study, we evaluated the bax,

bcl-2, gadd153, grp78, and AIF proteins involved in the apoptosis pathway. The results of our study showed that, D-pantothenic acid decreased bax, bcl-2 and grp78 protein expression in PC-3 prostate cancer cells but this decreased level did not statisticaly significant. The effect of D-pantothenic acid on gadd153 and AIF proteins in PC-3 prostate cancer cells was increased but this increased level did not statisticaly significant. Penthothenic acid had no effect on proapoptotic proteins and Anti-apoptotic protein bax, bcl-2, gadd153, grp78, and AIF. As a result, our study showed that pantothenic acid has no effect on intracellular apoptotic pathways.

As a conclusion our study showed that D-pantothenic acid had no effect on the growth of prostate cancer cells.

Yazar Katkıları: Çalışma konsepti/Tasarımı: AA; Veri toplama: AA, GÖ; Veri analizi ve yorumlama: GÖ; Yazı taslağı: AA; İçeriğin eleştirel incelenmesi: GÖ; Son onay ve sorumluluk: AA, GÖ; Teknik ve malzeme desteği: AA, GÖ; Süpervizyon: AA; Fon sağlama (mevcut ise): yok.
Etik Onay: Yazarlar, ticari olarak temin edilebilen insan hücre dizisi (İnsan prostat kanseri hücreleri PC-3 (ATCC® CRL-1435)) çalışmalarının bir etik kurul onayı gerektirmediğini teyit etmişlerdir..
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.
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Author Contributions: Concept/Design : AA; Data acquisition: AA, GÖ; Data analysis and interpretation: GÖ; Drafting manuscript: AA; Critical revision of manuscript: GÖ; Final approval and accountability: AA, GÖ; Technical or material support:AA, GÖ; Supervision: AA; Securing funding (if available): n/a.
Ethical Approval: The authors has confirmed that studies on commercially available human cell line (Human prostate cancer cells PC-3 (ATCC® CRL-1435) do not require an ethics committee approval.
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