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The Effects of Sulphanomide-E Derivative on Carnitine Metabolism of Cervix Cancer Cells

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Abstract

Introduction: Cervical cancer is one of the most common types of cancer in women worldwide. Recent studies have shown that metabolic programming can support cervical cancer treatment by increasing sensitivity to chemotherapy and radiotherapy. Therefore, the investigation of new treatment agents targeting the metabolism of cervical cancer is of great importance in terms of improving treatment outcomes and developing new strategies. In our previous studies, we determined that Sulfanomide-E derivative has an apoptotic effect on HELA cells. In this study, we aimed to investigate the effects of Sulfanomide-E on carnitine metabolism in HELA cells.

Method: HELA cells were used in our study and the cytotoxic and apoptotic effects of Sulfanomide-E were investigated. A dose of 25 µg/ml Sulfanomide-E was applied to HELA cells and the cells were incubated for 24 hours. In order to determine the changes in carnitine metabolism, a total of 27 carnitine and acylcarnitine derivatives were analyzed using LC-MS/MS. The obtained data were analyzed with SPSS 25.0 program.

Findings: As a result of the analyses, a significant increase was observed in all carnitine and acylcarnitine derivatives in the Sulfanomide-E applied groups compared to the control group. These findings indicate that Sulfanomide-E has an effect on carnitine metabolism and these metabolic changes may be important in terms of response to treatment.

Conclusion: This study reveals that Sulfanomide-E affects carnitine metabolism in HELA cells and exhibits apoptotic effects. The obtained data suggest that these changes in carnitine metabolism may provide a new understanding of the potential benefits of metabolic programming in the treatment of cervical cancer. Therefore, the role of agents such as Sulfanomide-E in the treatment of cervical cancer should be supported by more detailed studies.

Key words: Cervical cancer, carnitine, HELA cell line, sulfanilamide, LC-MS/M

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Sülfanomit-E Türevinin Serviks Kanser Hücrelerinin Karnitin Metabolizması Üzerindeki Etkileri

Öz

Giriş: Rahim ağzı kanseri, dünya genelinde kadınlarda en sık görülen kanser türlerinden biridir. Son yıllarda yapılan araştırmalar, metabolik programlamanın kemoterapi ve radyoterapiye olan duyarlılığı artırarak rahim ağzı kanseri tedavisini destekleyebileceğini ortaya koymuştur. Bu nedenle, serviks kanserinin metabolizmasını hedef alan yeni tedavi ajanlarının araştırılması, tedavi sonuçlarının iyileştirilmesi ve yeni stratejilerin geliştirilmesi açısından büyük bir önem taşımaktadır. Önceki çalışmalarımızda, Sülfanomid-E türevinin HELA hücreleri üzerinde apoptotik etki gösterdiğini belirlemiştik. Bu çalışmada ise Sülfanomid-E'nin HELA hücrelerinde karnitin metabolizması üzerindeki etkilerini incelemeyi amaçladık.

Yöntemler: Çalışmamızda HELA hücreleri kullanıldı ve Sülfanomid-E'nin sitotoksik ve apoptotik etkileri incelendi. 25 µg/ml Sülfanomid-E dozu HELA hücrelerine uygulanarak hücreler 24 saat boyunca inkübe edildi. Karnitin metabolizmasındaki değişiklikleri belirlemek amacıyla toplamda 27 adet karnitin ve asilkarnitin türevi, LC-MS/MS cihazı kullanılarak analiz edildi. Elde edilen veriler SPSS 25.0 programı ile analiz edildi.

Bulgular: Yapılan analizler sonucunda, Sülfanomid-E uygulanan gruplarda, kontrol grubuna kıyasla tüm karnitin ve asilkarnitin türevlerinde anlamlı bir artış gözlemlendi. Bu bulgular, Sülfanomid-E'nin karnitin metabolizması üzerinde etkili olduğunu ve bu metabolik değişikliklerin tedaviye yanıt açısından önemli olabileceğini göstermektedir.

Sonuç: Bu çalışma, Sülfanomid-E'nin HELA hücrelerinde karnitin metabolizmasını etkileyerek apoptotik etkiler gösterdiğini ortaya koymaktadır. Elde edilen veriler, karnitin metabolizmasındaki bu değişikliklerin, rahim ağzı kanseri tedavisinde metabolik programlamanın potansiyel faydalarına dair yeni bir anlayış sunabileceğini düşündürmektedir. Bu nedenle, Sülfanomid-E gibi ajanların serviks kanseri tedavisindeki rolü daha ayrıntılı araştırmalarla desteklenmelidir.

Anahtar kelimeler: Serviks kanseri, karrnitin, HELA hücre hattı, sülfanomid, LC-MS/MS

INTRODUCTION

Cervix cancer is one of the most commonly seen cancers in females worldwide. Some studies carried out recently revealed that metabolic programming can effectively support cervical cancer treatment by increasing chemo and radio sensitivity. As a result, the investigation of new agents which will affect the metabolism of cervix cancer is of the utmost importance in order to improve therapeutic result and develop new treatment strategies. This study was carried out to determine the effects of Sulphanomide-E derivative on HELA carnitine metabolism by which we determined that it showed apoptotic effect on HELA cells in our previous study. In our study, 25 µg/ml Sulphanomide dosage showing citotoxic and apoptotic effect on HELA cells was applied and incubated for 24 hours. Then 27 carnitine and acylcarnitine derivatives were analyzed by LC-MS/MS. The analysis of the obtained data was done with SPSS 25.0 program. At the end of the study, it was seen that there was an increase in all carnitine and acylcarnine derivatives in the Sulfanomide-E applied groups compared to the control group. According to the analysis results, the most significant increase between the two groups was determined to be C0, C2 and C14:2 and Sulfanomide-E derivative activated carnitine metabolism in HELA cells. We believe that this situation damages glucose metabolism in cervical cancer cells and causes them to obtain energy through fatty acids.

Cancer is one of the most significant health problems which threathen people's health and its incidence and growth poses serious threats for people[1]. In 2020, about 19.3 million new cancer cases were determined worldwide and about 10 million people died due to cancer[2]. The incidence of cervix cancer ranks fourth in females among cancer cases. It was determined

that in 2022, the incidence of new cases was 604.127 owing to cervix cancer and the number of deaths was 341.831[3]. The pathogenic factor of cervix cancer is HPV. HPV is a small enveloped DNA virüs which can be divided into low-risk and high-risk by natural disorder area and is the most common way of transmission for sexually-transmitted disease (STD)[4]. HPV virüs with common high risk are HPV 16 and HPV18. HELA belongs to HPV 18 cells[5]. Therefore, HELA cells have been used as a model to develop new drugs against cervix cancer.

Nowadays, radiotherapeutic and surgical methods including mainly chemotherapy have been used in the treatment of cancer[6]. However, the fact that chemotherapeutic agents are not confined to cancerous tissue leads to one of the most important factors which limit the treatment of cancer[7]. Hence, new studies have been conducted in order to determine the new molecules that are original to cancerous cells. In studies carried out recently, the expression of carbonic Anhidraz-IX (CAIX) especially in solid tumors allows this enyzme to be used as a biomarker and a target specific to cancerous cells in cancer treatment since it informs about the diagnosis and prognosis of cancer. Thus, detecting new molecules inhibiting Carbonic Anhidraz-IX can enable the emergence of new chemotherapeutic candidate drugs. It was determined that some molecules, especially sulphanomids, inhibited CAIX.

Sulphanomids have a number of biologic activities and it was determined that they showed anticancer activity in in-vitro and /or in-vivo 8. They form anticancer activity on various mechanisms and the most important one occurs through the inhibition of carbonic. L-carnitine is a vital molecule present in all living cells[9,10]. Its main function in cells of mammals is the oxidation of long-chained fat acids and the transfer of it along mitochondrial membrane for production of ATP energy[11]. Because of its main role as a basic group shuttle for fat acid oxidation of carnitine, it has an extremely significant role in cell metabolism. In physiological and pathological conditons where FAO takes place highly, the maintenance of carnitine hemoosthasis is extremely important for cell life[12].

Carnitine plays a vital role in the delivery of acyl groups across the intracellular membrane for FAO. Many human cancers rely on FAO to grow and become malignant. Moreover, carnitine is very important in the regulation of acyl CoA/CoA balance, which regulates carbohydrate and lipid metabolism[11]. In this study, it was investigated by LC-MS/MS method whether CA-IX inhibitor caused changes in the carnitine profile of HELA cells. Accordingly, it was investigated whether Sulfanomide-E agent affected the carnitine mechanism in its effect on cervical cancer.

METHOD

Cell Viability Assay

In this study, 25 M. dosage showing the most potent citotoxic and apoptotic effect on cervix cancer in our previous study was applied. IC50 value was determined as 13.7 M. Sulphanomide –E derivative used in this study was synthesized by Durgun et al. and CAIX inhibitor activity was determined. The anticancer activity of this agent in cervix cancer cells whose CAIX expression is high was studied by Temiz et al. In this study, the anticancer mechanism of sulphanomide-E derivative agent was examined for first time by studying its effect on carnitine metabolism.

Administration of Drugs To Cells And Examination of Carnitine Profile

After cells were reproduced in 75-cm-plate around 80%, 25 µg/ml dosage of sulphanomide-E derivative agent which showed the most potent cytotoxic and apoptotic effect on cervix cancer in our previous study was administered and incubated for 24 hours.

After incubation, medium was removed by tripsinization method.The removed cells were centrifuged for 1000 rpm in 5 minutes, and cold lysis buffer was added on pellet that occured. Cells homogenized using Tissueelyser were then centrifuged at 1000 rpm and supernatant was obtained. It was dripped into 5 mm gutria paper, dried at 25 C, cut by sonar and taken to clean tubes. Carnitine screening test was used. According to procedure, 200 µl internal was added on guitra paper and it was dried under nitrogen gas for 30 min. Having been dried,60 µl DRVT was added and left for incubation at 60 0C for 30 min and derivated and dried under nitrogen gas for 30 min. By adding 100 µl mobile phase solution, it was practised three repetitions by LC-MS/MS and the obtained data were evaluated.

Statistical Method

The conformity of the data to normal distribution was tested with Kolmogorow-Smirnov and Shaphiro Wilk tests. Independent Samples t Test was used for numerical variables showing normal distribution, and Mann-Whitney U test was used for independent two-group comparisons for those not showing normal distribution. As descriptive statistics, mean±standard deviation was given for numerical variables, and number and percentage values were given for categorical variables. SPSS Windows version 25.0 package program was used for statistical analyses and P<0.05 was considered statistically significant.

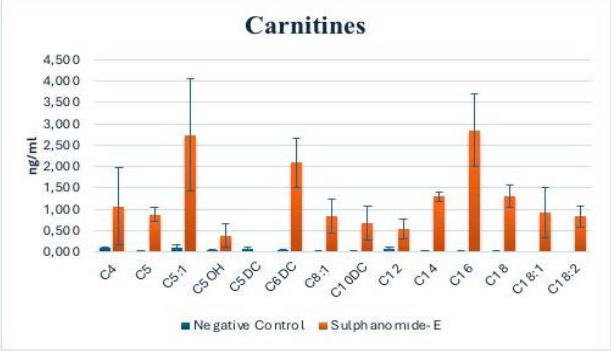
RESULTS

Table I: Statistical Analysis of Groups

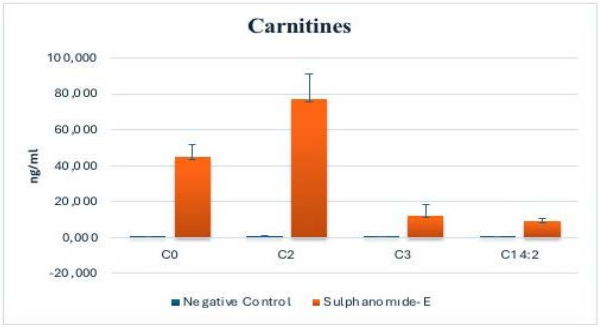
	Negative Control			Sulphanomıde-E (25 µg/ml)		
	Min	Max	Mean±SD	Min	Max	Mean±SD
C0	0,33	0,45	0,38 ± 0,05	38,42	56,09	44,69 ± 6,8
C2	0,81	1,05	0,94 ± 0,08	52,33	94,14	76,85 ± 14,41
C3	0,07	0,1	0,09 ± 0,01	5,36	22,17	12,01 ± 6,21
C4	0,08	0,11	0,09 ± 0,01	0,57	2,71	1,07 ± 0,89
C4DC	0,02	0,03	0,03 ± 0	0	1,44	0,76 ± 0,6
C5	0,02	0,03	0,03 ± 0	0,69	1,11	0,88 ± 0,17
C5:1	0	0,19	0,09 ± 0,09	1,42	4,54	2,74 ± 1,32
C5OH	0,03	0,06	0,05 ± 0,01	0,14	0,7	0,39 ± 0,26
C5DC	0,04	0,11	0,08 ± 0,03	0	0	0 ± 0
C6	0,03	0,04	0,03 ± 0	0	0,41	0,31 ± 0,17
C6DC	0,02	0,08	0,05 ± 0,02	1,27	2,78	2,09 ± 0,58
C8	0,01	0,02	0,02 ± 0	0	3,82	1,21 ± 1,48
C8:1	0,01	0,03	0,02 ± 0,01	0,3	1,28	0,84 ± 0,39
C8DC	0,05	0,07	0,06 ± 0,01	0	1,1	0,35 ± 0,52
C10	0,03	0,1	0,06 ± 0,03	0	0,67	0,5 ± 0,28
C10:1	0,02	0,05	0,03 ± 0,01	0	2,78	1,5 ± 1,03
C10DC	0,02	0,03	0,03 ± 0,01	0,27	1,15	0,69 ± 0,39
C12	0,03	0,12	0,07 ± 0,04	0,23	0,81	0,53 ± 0,24
C14	0,02	0,04	0,03 ± 0,01	1,12	1,46	1,3 ± 0,11
C14:1	0,01	0,04	0,02 ± 0,02	0	0,35	0,21 ± 0,14
C14:2	0,08	0,12	0,1 ± 0,01	7,53	11,92	9,02 ± 1,56
C16	0,02	0,03	0,02 ± 0	1,33	3,48	2,85 ± 0,85
C16:1	0,04	0,06	0,05 ± 0,01	0	2,86	1,28 ± 1,14
C18	0,01	0,02	0,01 ± 0,01	0,99	1,67	1,3 ± 0,26
C18:1	0	0	0 ± 0	0,32	1,78	0,93 ± 0,59
C18:2	0	0,01	0,01 ± 0	0,39	1,08	0,83 ± 0,26
C18:1 OH	0,01	0,03	0,02 ± 0,01	0	1,33	0,42 ± 0,63

*a. Mann-Whitney U Test, * p< 0.05, ** p<0.001 Negative (3 Repeats), Sulphanomide-E (3 Repeats)*

According to the results in Table 1, the statistical analysis of Sulphanomide-E compound according to the control group is given. According to this analysis, C0, C2, C3, C4, C5, C5:1, C5OH, C5DC, C6DC, C8:1, C10DC, C12, C14, C14:2, C16, C18, C18:1 and C18:2 values were found to be statistically significant (* $p<0.05$, **$p<0.001$).



Graph 1: Average Graph of Groups



Graph 2: Average Graph of Groups

DISCUSSION

Owing to important side effects of drugs which have been used in cancer chemotherapy, their therapeutic inadequacy and selectivity problems in their effects, the studies in this field have been continuing and taking attention since a treatment method providing a certain solution has not been fully developed yet. Currently, most of the research in cancer treatment focus on developing target-specific method and treatment techniques. In studies done recently, as it was determined that Carbonic-Anhidraz-IX enzyme showed that promising results for cancer diagnosis and treatment have appeared through the use of enzyme inhibitors. CAIX enzyme is a well-known transmembrane enzyme and while it is highly synthesized in most solid cancer types, it is synthesized in several normal tissues in a very limited amount. Therefore, having this characteristic makes CAIX be a new suitable target adress for cancer treatment in trems of clinical research[1,2].

It was reported that in various epithelial cells, the expression of CAIX in high levels is associated with Patient results such as breast cancer,non-small-cell lung cancer and cervix cancer. CAIX was firstly clonned from HELA cells in 1994 and it has been known that the only member related to tumour in CA family is CAIX. Later, Lieskovska et al. showed that CAIX had a role in cell-cell and cell-matrix interactions. Besides, due to a possible link between CAIX and HPV infection, it may be extremely significant to define CA9 expression in cervical cancer cells. Molecule can be used as a new guiding principle for diagnosing HPV infection. CAIX is an important component of tumoral pH regulatory system which may result from hypoxy[4].

As a result of its arrangement, a chemical buffer system was developed by hydrolizing CO2 into H+ and HCO3. While this helps intracellular pH to continue by being transferred into cell, it contributes as a H+ resorce for increasing acidosis in extracellular base. Thus, on one hand cancer cells are protected by damages of glicolitic mechanism, on the other hand, it shows metastatic activity thanks to extracellular acidosis medium[9,10]. Metastasis is the primary cause of death for more than 90% of cancer patients including human cervix cancer[3]. Hence, any intervention to be performed to one or more effectors is the evidence of producing an inhibitory effect on tumor growth. It was determined that CAIX halts the proliferation of cancer cells in in-vitro medium by various inhibitors (sulphanomide, coumarin etc.) and is effective in reducing

tumor growth and inhibits metastasis with no toxic effects which are not specific to various tumor models. In addition, if applied in combination with conventional chemotherapy and radiotherapy, it was revealed that such inhibitors inhibit the growth of various tumors[8]. It was also determined in previous studies that newly-synthesized aminosulphanomide derivative with CAIX inhibitory characteristic had cytotoxic effect on cervix cancer[11]. In this study, the role of carnitine metabolism in mechanism of action of sulphanomide agent was investigated. We determined that sulphanomide derivative E agent caused an increase in the level of carnitine and its derivatives on cervix cancer cells. As a result of the study, we already determined that sulphanomide E derivative agent resulted in cell death, leading to CAIX inhibition in cervix cancer cells. In this study, the effectiveness of CAIX inhibition on carnitine metabolism was studied. We also determined that there was a significant increase in the level of carnitine and its derivatives in cervix cancer cells. In normal conditions, although cancer cells regulate their metabolism through glucose, our agent caused TCA cycle to be activated, giving rise to an increase of carnitine level in cancer cell metabolism.

The metabolic flexibility in cancer cells is defined as its ability to respond to and adapt to metabolism in order to support and ensure the rapid proliferation of cells, sustained growth and surviving in hostile conditions. Cancer cells can rearrange/reprogramme their metabolism to preserve their integrity from harsh and hypoxic environment/medium as well as biosynthetic intermediate demand. Several studies have accepted these rescheduled activities as distinguishing features of cancer[11,12]. Recent findings point out that carnitine system may be regarded as a lacking in order to trigger The metabolic flexibility of cancer cells sensitively. In fact,the components of this system play a role in transport of main parts from cytozol to mitochondria,on the contrary,play a role in bidirectional transport,thus playing a vital role in regulating the transition between glucose and fat acid metabolism[12]. As a result, CS regulation plays a very significant role in tumors both in enzymatic and in epigenetic levels ,proposing new ways in prevention and treatment of human cancers. Carnitine is biosynthesized fro lysinand methionin acids and L-carnitine is biologically its active form[12]. It has been believed that carnitine carries long-chained achyl groups from fat acids to mitochondrial.

Matrix where they can be decomposed through B oxidation into achetil –CoA to obtain usable energy by way of citric acid cycle. Consequently, LC is necessary for producing metabolic energy in living cells. It has been well-known that most cancer cells produce energy with high glucose followed by lactic acid fermentation in cytosol, rather than low glucose rate following the oxidation of pyruvate in mitochondria as most cells do. This is known as Warburg's effect in cancer cells. Rapidly growing malignant cells have typically gliolitic speeds/rates about 200 times faster/higher than normal tissues of origin. Even though the Warburg effect has been challenged and developed more, this theory has been an evidence cited most frequently which states that tumors show dysfunctional metabolism. It has been known that citric cycle is inhibited in most cancer cells. In a study conducted, it was determined that carnitine results in disruption of cellular metabolism in cancer cells, however, it does not lead to any change in normal cells[11,12].

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It has been believed that carnitine carries long-chained achyl groups from fat acids to mitochondrial[25].

In conclusion, this study highlights the significant role of CAIX inhibition in cervical cancer cells, particularly in relation to carnitine metabolism. The findings suggest that the sulphanomide derivative E agent increases carnitine levels, triggering alterations in the TCA cycle, thereby influencing the metabolic flexibility of cancer cells. This metabolic adaptability, which is crucial for tumor proliferation and survival in hypoxic conditions, points to the potential of targeting carnitine metabolism as an innovative approach in cancer therapy. The increased understanding of CAIX's involvement in both pH regulation and carnitine metabolism opens new avenues for therapeutic strategies, especially when combined with conventional cancer treatments. Further research into the metabolic reprogramming of cancer cells could provide essential insights into more effective cancer therapies, particularly in overcoming metastasis and resistance mechanisms.

Ethics Committee Approval: Since the study was conducted as a cell culture, an ethics committee report was not required.

Conflict of Interest: The authors declared noconflicts of interest.

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