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ARAŞTIRMA / RESEARCH

A direct bridge to metformin and matrix metalloproteinase relationship in prostate cancer model: oxidative stress

Prostat kanseri modelinde metformin ve matriks metalloproteinaz ilişkisine doğrudan bir köprü: oksidatif stress

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Abstract

Purpose: The purpose of the study was to evaluate the effect of metformin as well as the possible role of Matrix metalloproteinase2 (MMP2) and oxidative stress parameters on the prostate cancer model.

Materials and Methods: Male Copenhagen rats were divided into three groups. Control group, cancer group, cancer+metformin (CM) group. 2x104 Mat-LyLu cells were inoculated subcutaneously to generate prostate cancer. Metformin treatment was administered daily by gavage following inoculation of the Mat- Lylu cells. The experiment was terminated on the 14th day following Mat-LyLu cell injection. Serum glutathione (GSH), prostate-specific antigen (PSA), and malondialdehyde (MDA) levels were determined by employing the Enzyme-Linked ImmunoSorbent Assay (ELISA) method. In addition, the serum matrix metalloproteinase (MMP) 2 activities were determined via ELISA.

Results: GSH was significantly increased in the CM group than in the cancer group. PSA, MDA and MMP2 were significantly lower in the CM group than in the cancer group. Oxidative stress parameters were significantly higher in the cancer group. Metformin reversed cancer's effect in GSH, PSA, MDA, and MMP2 parameters.

Conclusion: Prostate cancer model caused a detrimental effect on MMP and oxidative stress parameters, and metformin administration ameliorated the changes caused by cancer. Metformin showed its mechanism of action by inhibiting free radical products originating from prostate cancer and changing the antioxidant capacity. Metformin is a candidate to be a potential anticancer drug in the therapeutic cancer treatment process.

Keywords:. Matrix metalloproteinase, metformin, prostate cancer, experimental model, oxidative stress.

Öz

Amaç: Çalışmanın amacı, prostat kanseri modelinde metforminin etkisinin yanı sıra Matriks metalloproteinaz2 (MMP2) ve oksidatif stres parametrelerinin olası rolünü belirlemekti.

Gereç ve Yöntem: Erkek Copenhagen sıçanları üç gruba ayrıldı. Kontrol grubu, kanser grubu, kanser+metformin (CM) grubu. Prostat kanseri, 2x104 Mat-LyLu hücrelerinin subkutan enjeksiyonu ile oluşturuldu. Metformin tedavisi, Mat- Lylu hücrelerinin ardından gavaj yoluyla günlük olarak uygulandı. Mat-LyLu hücre enjeksiyonunu takiben 14. günde deney sonlandırıldı.. Enzyme Linked Immuno Sorbent Assay (ELISA) yöntemi kullanılarak serum glutatyon (GSH), prostat spesifik antijen (PSA) ve malondialdehit (MDA) düzeyleri belirlendi. Ayrıca serum matriks metalloproteinaz 2 (MMP) 2 aktiviteleri ELISA aracılığıyla belirlendi.

Bulgular: GSH, CM grubunda kanser grubuna göre önemli ölçüde arttı. PSA, MDA ve MMP2, CM grubunda kanser grubuna göre anlamlı derecede düşüktü. Oksidatif stres parametreleri kanser grubunda anlamlı olarak yüksek bulundu. Metformin GSH, PSA, MDA ve MMP2 gibi parametrelerdeki kanser etkisini tersine döndürdü.

Sonuç: Prostat kanser modelinin MMP ve oksidatif stres parametreleri üzerine zararlı bir etkiye neden olduğunu ve metformin uygulamasının da kanserin neden olduğu değişiklikleri iyileştirdiği saptandı. Metforminin etki mekanizmasını prostat kanseri kaynaklı serbest radikal ürünlerini inhibe ettiği ve antioksidan kapasiteyi değiştirerek gösterdiği belirlendi. Metformin, terapötik kanser tedavi sürecindeki potansiyel bir anti kanser ilacı olmaya adaydır.

Anahtar kelimeler: Matriks metalloproteinaz, metformin, prostat kanseri, deneysel model, oksidatif stress.

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INTRODUCTION

Cancer and noncommunicable diseases are now recognized as a global threat. It is thought that 75% of the world's predicted 21 million new cancer and 13 million cancer deaths annually by 2040 will seriously increase the cancer burden, especially in low and middle-income countries. Cancer research is still of great importance in every aspect[1].

Despite extensive public awareness campaigns and considerable advancements in diagnosis, screening, and treatment methods, prostate cancer continues to be the second leading cause of cancer-related death in men worldwide[2].

The extracellular matrix (ECM) not only supports organs and tissues but also plays a role in cell cycle and cell motility, survival, and apoptosis, as well as growth factor distribution and integration. MMP are extracellular proteases that play an important role in physiological and pathological tissue destruction. They belong to a 23 members endopeptidase family that consists of zinc, calcium bound, and remodel the proteins that make up the ECM[3].

Matrix metalloproteinases (MMP) have long been involved in cancer cell invasion and metastasis since MMP has a strong influence on tumor ECM remodeling. MMPs have important roles in cancer invasion by inducing genomic instability and DNA damage[4]. In cancer physiology, invasion, and metastasize situations, ECM must be destroyed. MMPs are synthesized by epithelial and mesenchymal cells. MMP9 and MMP2, which are most frequently detected in malignant tissues and found to be associated with tumor and metastatic potential[5].

The ECM plays a crucial role in regulating the tissue microenvironment and maintaining cellular homeostasis. Many proteins can remodel the ECM by promoting tumor invasive processes[6].

Type 2 diabetes and the mechanism of cancer share many common risk factors. In particular, common links such as hyperinsulinemia, chronic inflammation, obesity, and metabolic syndrome promote these diseases more. Individuals with type 2 diabetes have a greater incidence and mortality rate than those with tumors of the digestive system. Metformin has been used for many years to treat type 2 diabetes. Importantly, patients with diabetes on long-term metformin use have a lower incidence of tumors and cancer-related mortality. Moreover, recent research shows that metformin may have direct anticancer activity[7]. Metformin is currently the ideal candidate for antineoplastic action. Firstly, metformin can directly activate AMP-activated protein kinase (AMPK). Secondly, metformin can decrease activation of the IGF receptor signaling[8].

Experimental animal models are used in research to establish the diagnosis of various diseases and to develop treatment methods. Our study hypothesizes that oxidative stress markers could be increased in the prostate cancer model. Because the cancer process affects reactive oxygen species (ROS) production. On this basis the aim of our study, we aimed to reveal the damage in the prostate cancer model and whether metformin, an antidiabetic drug, has a therapeutic effect on these damages, for the first time, with an in vivo experimental model.

MATERIALS AND METHODS

Cell culture

Dunning model highly metastatic Mat-LyLu cells were grown in RPMI culture medium (RPMI-1640, Gibco; Life Technologies, USA), supplemented with 1% fetal bovine serum (FBS) (Gibco), 2 mM L-glutamine (Gibco; Life Technologies, USA), and 250 nM dexamethasone (Sigma; Sigma-Aldrich, USA). Cells were maintained in a 37 ^{o}C/5% CO_2 incubator [9,10].

The study was approved by the Animal Care and Use Committee of the Istanbul University, Istanbul, Turkey (the ethic no: 2014/28, date: 27.02.2014).

Experimental design

Male Copenhagen rats from the "Tubitak Mam Genetical Engineering and Biotechnology Institute" were kept under controlled conditions of humidity (65%–70%), temperature (22°C±2°C), and under standard light/dark (12 hour/12 hour) cycles. The entire experiment process was completed at the Istanbul University Experimental Medicine Research Institute (DETAE). I am thankful to Dr. Ilknur Bugan for the prostate cancer (Mat-Lylu process) experimental period. I applied all other injections to Copenhagen rats. We selected these rats because these cell lines were special for them to create a prostate cancer model. Copenhagen rats were divided randomly into three groups. In the project study, the use of at least 8 subjects for the control group, cancer, and treatment groups were calculated by performing power analysis with the pass 2008

program, to create a Dunning cancer model at α=0.05 significance level, to obtain 0.8% power.
Copenhagen rats were randomly selected and divided into three groups of eight rats control (physiological saline), cancer, and CM groups. The $2x10^4$ Mat-LyLu cells were applied to the rats for treating prostate cancer. Metformin (250 mg/kg) (SIGMA, D150959-5G) 0.2 ml PS was applied. On the 14th day, the animal study came to an end to obtain early prostate cancer (Figure 1)[11]. Groups were sacrificed under ketamine hydrochloride (Ketalar®, Eczacıbaşı) and xylazine HCl (Alfazyne®, Holland) anesthesia. The body weight was measured. The sera were stored at -86 °C until required for use.

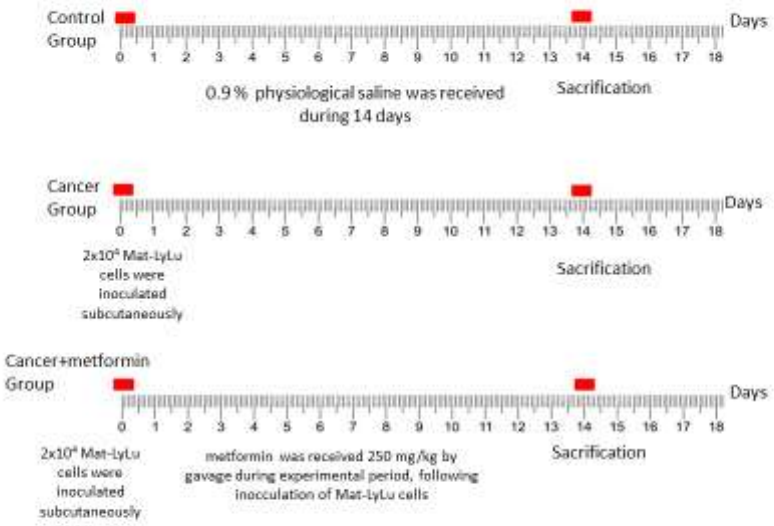

Figure1. Experiment procedure and treatment schedule.

Biochemical analysis

Serum was obtained from the blood samples by centrifugation for 15 minutes at 3000 rpm. Serum levels of GSH, PSA, MDA, and MMP2 were measured using an enzymatic kit procedure. Serum levels of GSH (Sunred201-11-5134), PSA (Sunred-201-11-0559), MDA (Sunred-201-11-0157), MMP2 (ab213910) were estimated using commercial enzyme-linked immunosorbent assay kits namely according to the manufacturer's instructions.

Statistical analysis

Statistical significance was analyzed using Prism v.6 (GraphPad Software). Statistical analysis of biochemical data was done by OneWay ANOVA. Mean ±standard deviation (SD) was used for result expression. The differences between groups were determined with Tukey's multiple comparisons test. The significance of differences was taken as the level of $p<0.05$.

RESULTS

GSH decreased ($P < 0.01$) in cancer compared to the control group. The GSH levels were low in cancer and increased significantly ($P < 0.05$) in the CM group. PSA reached the highest value ($P < 0.01$) in the cancer group. However, PSA decreased to a level similar to the control group in the CM group. MDA levels increased ($P < 0.01$) in all groups compared to the control group (Table 1). MDA levels increased in

cancer, decreased ($P > 0.05$) with metformin application. The serum MMP2 levels of the cancer group were notably higher compared with the control group. It was decreased ($P < 0.01$) following metformin administration.

Table 1. Serum GSH, PSA, MDA and MMP2 levels of experimental groups (± SD)

Groups	GSH (mg/L)*	PSA (pg/mL)*	MDA (nmol/mL)*	MMP2 (ng/mL)*
Control	320.43±7.30	712.77±44.35	9.87±0.68	20.40±3.3
Cancer	229.91±10.96a	1069.23±62.77a	13.46±0,70a	30±4.32 a
CM	283.67±10.70a,b	704±23.61b	12.53±0.62a	25.58±4.42 a,b
PANOVA	0.0001	0.0001	0.0008	0.003

a: $P < 0.01$ versus control group, b: versus cancer group.
GSH: Glutathione, PSA: Prostate-specific antigen, MDA: Malondialdehyde: MMP2: Matrix metalloproteinase 2, CM: Cancer+metformin

DISCUSSION

Metastasis means that cancer spreads to a different body part. The main reason why cancers result in death is the occurrence of a history of metastasis. In the process called metastasis, the formation of new cancer points from the primary cancer focus via blood and lymph is possible, and cancer may develop in different regions. They do this by using MMP that degrade the ECM. Especially considering that MMPs are inflammation markers, their effect on the cancerization process is inevitable[12]. Metformin, which is used as a type 2 diabetes treatment agent, has been known to slow down and sometimes stop carcinogenesis in recent years[13]. Prostate cancer seems to be the top cause of male death in the coming years. Considering that early diagnosis saves lives, solving many links of this disease will make it easier to understand the prostate cancer mechanism. The effect of metformin on prostate cancer is known recently but its mechanism of action has not been fully elucidated yet[14]. Considering these parameters together, it is aimed to investigate and clarify the relationship between MMP, metformin, and prostate cancer.

MMPs acts a vital role in a variety of biological functions, including many features of the immune response. MMPs may also act on proinflammatory cytokines and chemokines[15]. MMPs play a vital role in the classic hallmarks of cancer, including migration, invasion, metastasis, and angiogenesis[16].

MMPs are related to tumor aggressiveness and overall survival and are used as markers of malignancy. The present results suggest that MMP2 expression is a potential marker for prostate tumors after prostatectomy[17].

Metformin is commonly used in enhancing liver insulin sensitivity. Recent studies of metformin therapy in osteoarthritis focus on its anti-inflammatory and cartilage matrix protecting effects. Metformin has emerged as a potential anticancer agent[18].

ROS affects cancer induction[19]. Oxidative stress mitigates cancer development. It is accepted that chronic inflammation may lead to carcinogenesis[20]. Studies have shown that ROS levels increase and GSH levels decrease during the cancer process[21]. It has been determined that serum levels of MDA increase progressively in patients with colorectal cancer and reach the highest value in the fourth stage of cancer[22]. ROS, an inevitable by-product of cellular metabolism, exerts beneficial effects by regulating signaling cascades and homeostasis. It shows that prostate cancer is closely related to age and that elevated ROS levels are mediated through activation of the signaling pathway, which facilitates prostate cancer initiation, development, and progression[23].

Metformin has been found to have antiproliferative and anticancer effects in many solid tumors, including prostate cancer[24]. The direct anticancer mechanism of metformin is thought to activate protein kinases activated by liver kinase B1 and adenosine monophosphate, inhibit the activity of rapamycin and induce apoptosis and autonomy through p53 and p21[25]. Thus, the reduction in prostate cancer risk and metformin may be the result of the drug's direct effects of anticancer mechanisms or secondary effects resulting from amelioration of the metabolic syndrome. Especially, it was found that metformin is used as an adjuvant antineoplastic agent in cancer models[26].

There are only a few studies that affect serum levels of MMP2, oxidative stress parameters, metformin, and prostate cancer model. The study has several limitations. The main limitation in this study is our small conceptions.

The present study was limited by the limited sample size. Other possible limitations of this study included biochemical parameters. Future studies are needed for mechanical properties. Developing metformin's potential for the prevention or treatment of human prostate cancer. Hence, additional parameters should be examined to provide valuable data and utilize the appropriate histological and other biochemical results. As mentioned earlier, metformin is widely used as a biguanide agent in diabetes for insulin resistance. Recently, metformin affects anticancer mechanisms. The present findings show that metformin has ameliorative effects against damage and changes in the total oxidant showed a beneficial effect. MMP -cancer- metformin correlation studies are necessary to gain more information about the subject. The results in this study may serve as preliminary data for further studies to elucidate the mechanism of metformin and MMP2 action.

This study suggested that further studies should be carried out to reveal unknown details regarding the ameliorative effect of metformin on oxidative stress exposed to prostate cancer toxicity.

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