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Original Article / Özgün Araştırma

Adipose Tissue Mesenchymal Stem Cells Exposed To Oxytocin and Sunitinib are Synergistically Dystrophic

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Abstract

Objective: Mesenchymal stem cells (MSCs) are also promising in immunosuppressed patients after organ and tissue transplantation, in addition to their current wide range of uses and research areas. Sunitinib is a receptor tyrosine kinase with immunosuppressive properties and its cytotoxic activity in different types of cells is known. Our study aimed to elucidate the effect of oxytocin on sunitinib-treated MSCs.

Methods: For this purpose, commercially available rat adipose tissue-derived MSC (ADMSCs) was used. The individual or combinational effect of the active substances on viability was evaluated with WST-1, the effect on apoptosis Annexin V, the effect on oxidative stress markers MDA, CAT, GPX, and SOD ELISA tests.

Results: The IC50 value of sunitinib was determined as 44.57 µM at the 48th hour, and it was determined that oxytocin had no cytotoxic effect in doses up to 100 µM. Treatment of the two agents in combination increased the cytotoxic effect of sunitinib. Oxytocin attenuated the effect of sunitinib on apoptosis and lipid peroxidation.

Conclusion: It is important to investigate the efficacy of these two substances individually and in combination with ADMSCs with further experiments to evaluate the potential use of oxytocin in organ and tissue transplantations.

Keywords: Mesenchymal Stem Cells, Oxytocin, Sunitinib

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Oksitosin ve Sunitinib'e Maruz Kalan Yağ Dokusu Mezenkimal Kök Hücreleri Sinerjistik Olarak Distrofiktir

Öz

Giriş: Mezenkimal kök hücreler (MKH) mevcut geniş kullanım ve araştırma alanlarına ek olarak, organ ve doku nakli sonrası immün sistemi baskılanmış hastalarda da umut vadetmektedir. Sunitinib, immun baskılayıcı özelliği de olan bir reseptör tirozin kinazdır ve farklı tipteki hücrelerde sitotoksik etkinliği bilinmektedir. Çalışmamızda oksitosinin sunitinibin uygulanmış MKH'ler üzerindeki etkisinin aydınlatılması amaçlanmıştır.

Yöntemler: Bu amaçla ticari olarak temin edilmiş sıçan adipoz doku kökenli MKH (ADMKH) kullanılmıştır. Etken maddelerin bireysel ve kombinasyon halinde canlılık üzerine etkisi WST-1, apoptoz üzerine etkisi Annexin V, oksidatif stres belirteçleri üzerine etkisi MDA, CAT, GPX ve SOD ELISA testleri ile değerlendirilmiştir.

Bulgular: Sunitinibin IC50 değeri 48. saatte 44,57 µM olarak belirlenmiş, oksitosinin 100 µM'a kadar doz uygulamasında sitotoksik etkiye sahip olmadığı belirlenmiştir. İki maddenin kombinasyon halinde uygulanması, sunitinibin sitotoksik etkisini arttırmıştır. Oksitosin, sunitinibin apoptoz ve lipid peroksidasyonu etkisini hafifletmiştir.

Sonuç: Bu iki maddenin bireysel ve kombinasyon halinde ADMKH üzerindeki etkinliğinin ileri deneylerle araştırılması, oksitosinin organ ve doku nakillerinde potansiyel kullanımının değerlendirilebilmesi açısından önem taşımaktadır.

Anahtar kelimeler: Mezenkimal Kök Hücre, Oksitosin, Sunitinib.

INTRODUCTION

Although organ and tissue transplantation is a popular approach in contemporary medicine, rejection by the host is a frequent complication encountered after transplants[1]. Immunosuppressive drugs are utilized in transplantation procedures to inhibit the host's immune reaction to the transplanted graft tissues[2]. Sunitinib is a small molecule receptor tyrosine kinase inhibitor that is used in the therapy of different cancers. It has also an immunomodulatory effect mechanism[3]. Mesenchymal stem cells (MSC) are a type of adult stem cell that may self-renew and differentiate[4]. MSCs are widely utilized in research since it is readily accessible from a variety of sources and has no ethical constraints[5]. Because of their self-renewal and differentiation capacity, MSCs are often used in regenerative medicine for tissue repair[6]. Since MSCs have immunosuppressive properties, they are given to the recipient along with the chemical agents applied during transplantation[7]. Oxytocin, a neuropeptide hormone, participates in numerous physiological and pathological processes like birth, maternal behavior, social behavior[8,9]. In stem cells, oxytocin regulates cell development, cell differentiation and improves cell viability[10].

In the light of the background information, this study aims to illuminate the effects of oxytocin on the sunitinib exposed MSCs viability, apoptosis, and oxidative stress mechanisms.

METHODS

Cell Culture Procedure

The rat adipose tissue-derived MSC line (ADMSC; Millipore, Merck) was commercially obtained. The cells were cultured in DMEM/F12 media (Cat. No: 11320033) with 1% penicillin/streptomycin, 1% L-glutamine, and 10% FBS (Cat. No: 10270-106). The cells were cultivated in the 75 cm2 flasks (Cat. No: 430639, Corning)at 37OC in an incubator (Class 100 from Thermo Electron Corporation) with 95% humidity and 5% CO2. The fifth passage cells were used in all experiments.

Cytotoxicity Assay

The cells were plated at 5×104 cells/ ml concentration to determine the IC50 values of the sunitinib and oxytocin. After 24 h incubation

period the medium was removed. Sunitinib and oxytocin were treated to the cells at a dosage range of 1 nM to 100 µM, with a final volume of 100 µl in each well. The cells with no drug were used as the control group. Formazan dye formation was assessed photometrically by adding 10 µl of WST-1 solution (Cat.No: 11644807001, Roche) to each well 24, 48, and 72 h after the active compound treatment, and measuring it every 15 min with a Multiscan FC (Thermo) microplate reader at 420 nm wavelength. GraphPad (Prism) was used to calculate the IC50 value of sunitinib and oxytocin on rat ADMSC.

Combination Assay

The cells were plated at 5×104 / ml concentration to investigate the probable synergistic, additive, or antagonistic effects of the combinations of sunitinib and oxytocin. The cells were incubated for 24 h at 37oC, 5% CO2 before the medium was removed. The sunitinib and oxytocin were treated to the cells at the dose values that determined by cytotoxicity assay. The cells with no active chemicals were used as the control group. Formazan dye formation was assessed photometrically by adding 10 µl of WST-1 solution (Cat.No: 11644807001, Roche) to each well, and measuring it every 15 min with a Multiscan FC (Thermo) microplate reader at 420 nm wavelength. CalcySyn software was used to calculate combination index of sunitinib and oxytocin on rat ADMSC.

Annexin V Assay

The FITC Annexin V Apoptosis Detection Kit I (Cat. 556547, BD) and The BD Accuri C6 flow cytometer were used for apoptosis analysis. The cells were plated at 3×105cell/ ml concentration in 6-well plates and they were incubated for 24 h. Sunitinib and oxytocin were treated to the cells at the IC50 dosages determined in the WST-1 test. Following the drug treatment, the cells were incubated for 48 h. At the end of the 48 h incubation period, the Annexin V protocol was applied according to manufacturer's protocol and the early-late apoptosis, total apoptosis, and dead cells were determined using BD Accuri C6 flow cytometer.

Measurements of Oxidative Stress Analysis Parameter

Antioxidant effects of the oxitocin and sunitinib were assessed using lipid peroxidation (MDA), glutathione peroxidase (GSH-Px), catalase activity (CAT), and superoxide dismutase (SOD) activity measurements by using Lipid Peroxidation (MDA) Colorimetric/Fluorometric Assay Kit (Cat. No: K739-100, BioVision), Glutathione Peroxidase Assay Kit (Cat. No. K762-100, BioVision), Catalase Assay Kit (Cat. No. K773-100, BioVision), and Superoxide Dismutase (SOD) Activity Assay Kit (BioVision, Cat. No. K335-100), respesctively. All oxidative stress indicator tests were carried out according to the manufacturer's instructions, utilizing 1x106 cells per milliliter for each dosage and control group. All measurements were taken using a Multiscan Elisa Plate Readers instrument at 532 nm, 340 nm, 570 nm, and 450 nm.

Statistical Analysis

IBM SPSS Statistics 23.00 (Statistical Package for Social Sciences) software was used for statistical analyses. In the case of continuous variables, the mean, standard deviation and error, minimum and maximum values, and 95 percent confidence intervals for the means are shown. Because the interaction between the two variables was significant, four groups were created and one-way analysis of variance was used. For pairwise analysis of groups after multiple comparisons, the Dunnett T3 test was used. All hypothesis tests were carried out at the 0.05 significance level.

RESULTS

The Cytotoxic Effects of Sunitinib and Oxytocin on ADMSC

The IC50 value of the sunitinib was determined as 44.57 µMat the 48th hour using GraphPad (Prism) software. Oxytocin had no cytotoxic effect on cells at doses of up to 100 µM (Figure 1). When the combinational effect of sunitinib and oxytocin was investigated by WST-1 test and CalcuSyn software, it was determined that the oxytocin treatment decreased the IC50 dose of sunitinib to 8.30 µM (Figure 2).

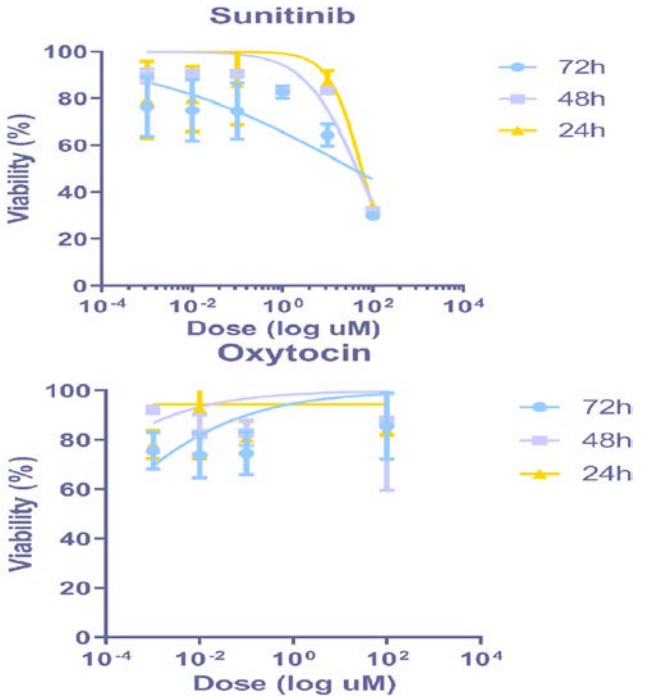

Figure 1: The cytotoxic effects of sunitinib **(a)** and oxytocin **(b)** in ADMSCs.

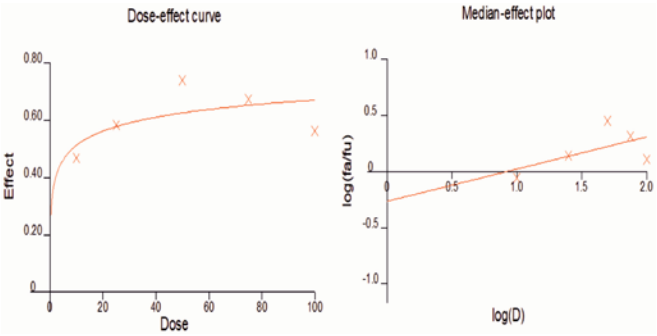

Figure 2: Combination effects of sunitinib and oxytocin in ADMSCs.

Apoptotic Effect of Sunitinib and Oxytocin on ADMSCs

Annexin V test results were shown in Figure 3. The percentage of dead and live cells were 1.03% and 98.97% in the control group, respectively. In the sunitinib group, the early apoptosis, late apoptosis, dead, and livecell percentages were 7.69%, 4.31%, 8.10%, and 79.90%, respectively. In the oxytocin group, the dead and live cell percentages were 2.59% and 97.41%, respectively. Combination group results, the rate of early apoptosis was 3.90%, the rate of late apoptosis was 1.34%, the percentage of dead cells was 7.81%, and the percentage of living cells was 25.5%. All experimental groups were shown microscopically after Annexin V staining. The number of cells stained was found to be high in the Sunitinib group (Figure 4).

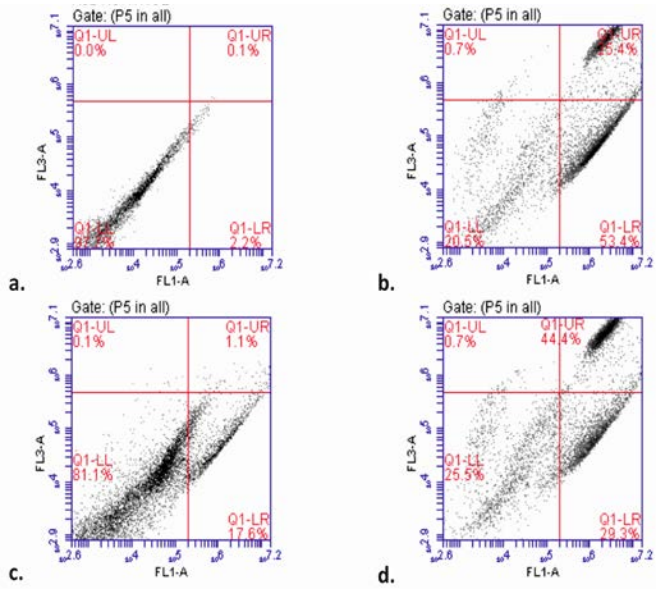

Figure 3: Annexin V test flow cytometry results of control (**a**), sunitinib (**b**), oxytocin (**c**), and combination (**d**) groups in ADMSCs. Live (LL), necrotic (UL), late apoptotic (UR), and early apoptotic cells (LR) were shown.

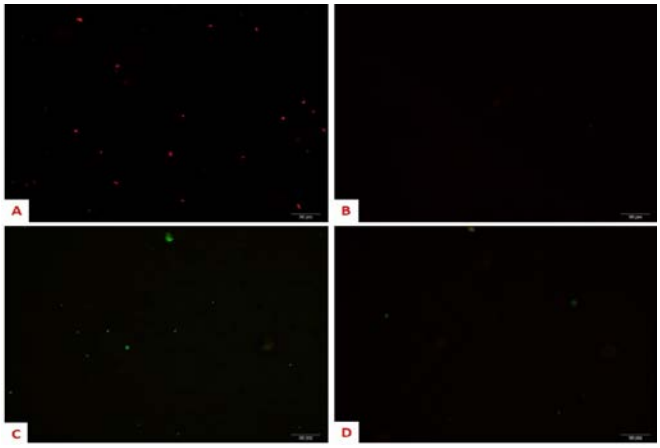

Figure 4: Microscopic representation of Annexin V staining of control (**a**), oxytocin (b), sunitinibn (**c**), and combination (**d**) groups in ADMSCs.

Viability Measurements of Combination Groups on ADMSCs

The statistical analyses of the viability measurements of the cells were shown in Figure 5. The difference between all comparisions was significant ($p<0.05$) in the necrotic cells, expect sunitinib-combination groups. The difference between the control-sunitinib, control-combination, oxytocin-sunitinib, oxytocin-combination, and sunitinib-combination groups was significant ($p<0.05$) in the late apoptotic cells.The difference between the control-sunitinib, control-combination, oxytocin-sunitinib, oxytocin-combination and sunitinib-combined groups was significant ($p<0.05$) in the early apoptotic cells. The difference between all comparisions was significant ($p<0.05$) in the lived cells.

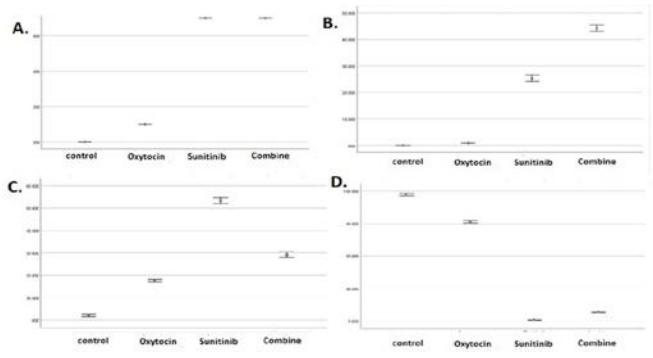

Figure 5: Statistical graphic 95% CI of viability measurements of ADMSCs. Necrotic (**a**), late apoptotic (**b**), early apoptotic (**c**), and live (d) cells.

Oxidative Stress Parameter Measurements of Combination Groups on ADMSCs

The results of the oxydative stress parameters results were shown in Figure 6. The difference between oxytocin-sunitinib, oxytocin-combination, and sunitinib-oxytocin groups was significant ($p<0.05$) in the MDA levels. The difference between control-oxytocin, control-combination, and oxytocin-combination groups was significant ($p<0.05$) in the GPX levels. The difference between oxytocin-combination and control-combination groups was significant ($p<0.05$) in the CAT levels.The difference between control-oxytocin, control-combination, and control-sunitinib groups was significant ($p<0.05$) in the SOD levels.

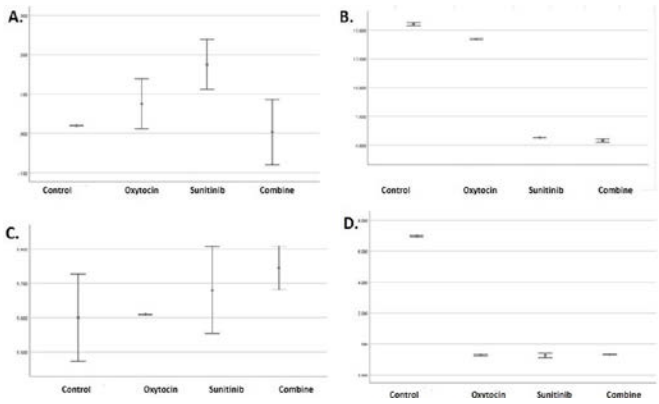

Figure 6: Statistical graphic 95% CI of oxidative stress parameter measurements of ADMSCs. MDA (**a**), GPX (**b**), CAT (**c**), and SOD (**d**) levels.

DISCUSSION

In this study, the effects of sunitinib, an immunosuppressive drug used in organ and tissue transplantations, on rat ADMSC and the potential of oxytocin to minimize the harmful effects of sunitinib were investigated.

Sunitinib is a small molecule receptor tyrosine kinase inhibitor used in the treatment of cancer. Sunitinib has been shown to suppress tumor growth when administered orally to animals following tumor formation in vivo studies. It is suggested that this process occurs through the supperession of VEGFR and PDGFR receptors[11].

Sunitinib has also an immunomodulatory effect mechanism[3].

There is no research on the harmful effects of sunitinib on mesenchymal stem cells in the literature. However, imatinib, a tyrosine kinase inhibitor comparable to sunitinib, has been demonstrated in research to decrease the growth of human mesenchymal stem cells[12].

Studies have demonstrated that ADMSCs can both in vitro and in vivo enhance healing rates and shorten the recovery period[6]. ADMSCs stimulate angiogenesis, fibroblast development and migration, fibronectin and collagen synthesis, and the release of growth factors and cytokines to particular cell lines such as keratinocytes, fibroblast-like cells, and endothelial cells. Furthermore, through IL-10 production and activity, as well as T-cell activation, these adipose-derived cells assist maintain the immune system's equilibrium[13,14].

Oxytocin causes several cell types to resistant to oxidative damage. Oxytocin treatment also regulates stem cell differentiation. As a result, oxytocin treatment may have significant ramifications in cellular applications[15]. Kim and colleagues realized a study in which human MSC was prepared with oxytocin improves heart healing and enhances cardiac function in a rat model with ischemia/reperfusion damage[16]. A functional study of oxytocin-induced MSC by Noiseux and colleagues indicated enhanced cellular proliferation, cell protection against the cytotoxic and apoptotic effects of hypoxia and serum deprivation, and cellular migration[17].

Studies are showing that sunitinib significantly inhibits the viability of cells. Although it has been reported to have toxic effects on cells both in vivo and in vitro, there is no IC50 value determined for ADMSC exposed to sunitinib in the literature. In our study, the IC50 dose of sunitinib was determined at the 48th hour, suggesting that sunitinib has a time-dependent manner effect on the viability of ADMSCs.

Current research focuses on evidence that oxytocin, which is utilized as an antioxidant, decreases cell damage. In stem cells, oxytocin treatment regulates cell development. Oxytocin is essential in cellular therapy applications because it can help by improving the host cell environment and promoting healing by changing the cell response. Oxytocin and MSC studies have revealed that the oxytocin osteoblast/adipocyte balance is controlled. Furthermore, it has been discovered that oxytocin therapy increases cell survival and proliferation[18].

In our study, it was determined that oxytocin did not have a cytotoxic effect on the rat ADMSC line at the dose range up to 100 μM.

As a result of the literature review, there are findings that oxytocin increases cell proliferation. Although it was initially suggested that oxytocin would prevent the cytotoxic effect of sunitinib by increasing cell proliferation in MSCs, it was determined that this combination enhanced the sunitinib cytotoxic effect. The IC50 dose of sunitinib decreased to 8.30 μM, and it was determined that the two substances had synergistic cytotoxic effects.

There are studies in the literature in which sunitinib was used as a cytotoxic agent. There are studies in which oxytocin is used as an antioxidant substance[19]. However, there is no study in which the two substances are used together. This situation enables our study to bring new information to the literature.

In line with the lipid peroxidation assay results, we observed that the use of sunitinib alone creates oxidative stress, the use of oxytocin alone reduces oxidative stress, and there is a decrease in the combination group. In terms of glutathione peroxidase parameters, it was observed that the use of sunitinib alone caused oxidative stress, and the use of oxytocin alone reduced oxidative stress, but there was no significant effect in the combination group. In

terms of catalase parameters, the expected effect was not observed. Interestingly the oxytocin and sunitinib combination significantly increased the catalase activity. We observed that oxytocin has no significant effect on the SOD parameters.

It is thought that the use of oxytocin and ADMSC may have positive effects on the patients which under immunosuppressive treatment following the tissue and organ transplantations.

Although oxytocin is an important potential agent for clinical applications, its usage in combination with sunitinib may cause an unexpected cytotoxic effect on MSCs. It is especially important to investigate the underlying mechanisms that cause these results. As the results that will be obtained in further studies on this combination, it is important in terms of the usage of oxytocin in clinical studies and cellular therapies.

Ethics Committee Approval: This manuscript does not contain any studies with human participants or animals.

Conflict of Interest: The author declares no conflict of interest.

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