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Review
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Can mesenchymal stem cells ameliorate testicular damage? Current researches

Mezenkimal kök hücreler testis hasarını iyileştirebilir mi? Güncel çalışmalar

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Abstract

Many recent studies have demonstrated the therapeutic effects of mesenchymal stem cells (MSC) in different disease models. Infertility is a global disease with a high prevalence. Non-obstructive azoospermia may occur due to genetic factors, exposure to toxic substances, anticancer treatments such as radiotherapy and chemotherapy and testicular torsion. Many experiments have been conducted to determine the efficacy of MSCs in the treatment of male infertility due to their differentiation capacity and paracrine effect. In these studies, the differentiation capacities of MSCs, obtained from diverse sources, to male germ cells were determined in vitro and their effects on testis niche were assessed by injection of MSCs into the testis. In this review, we addressed a few of the causes of non-obstructive azoospermia and summarized the current studies to determine the therapeutic effects of MSCs on testicular injury.
Keywords: Azoospermia, Male infertility, Mesenchymal stem cells, Stem cell therapy, Testicular damage

Öz

Son zamanlarda yapılan birçok çalışma, farklı hastalık modellerinde mezenkimal kök hücrelerin (MKH) terapötik etkilerini göstermiştir. İnfertilite, yüksek prevalansa sahip global bir hastalıktır. Obstrüktif olmayan azospermi, genetik faktörler, toksik maddelere maruz kalma, radyoterapi, kemoterapi gibi antikanser tedavileri ve testis torsiyonu nedeniyle ortaya çıkabilir. MKH'lerin farklılaşma kapasiteleri ve parakrin etkileri nedeniyle erkek infertilitesinin tedavisinde etkinliğini belirlemek için birçok çalışma yapılmıştır. Araştırmalarda, farklı kaynaklardan elde edilen MKH'lerin erkek germ hücrelerine farklılaşma kapasiteleri in vitro olarak belirlenmesinin yanı sıra bu hücrelerin testis nişi üzerindeki etkileri MKH'lerin testis içine enjekte edilmesiyle belirlenmiştir. Bu derlemede, obstrüktif olmayan azosperminin nedenlerinden birkaçına değindik ve MKH'lerin testis yaralanması üzerindeki terapötik etkilerini belirlemek için mevcut çalışmaları özetledik.
Anahtar kelimeler: Azospermi, Erkek infertilitesi, Mezenkimal kök hücreler, Kök hücre tedavisi, Testis hasarı

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Introduction

Infertility is a global public health problem, characterized by failure to achieve pregnancy after 12 months of unprotected sexual intercourse, which affects about 10-15% of couples worldwide. The contribution of the male factor is estimated to be 50-60%. The male factor alone is responsible in 20% of couples who cannot conceive [1-4]. Environmental, physiological, and genetic factors are effective in male infertility. Environmental factors include smoking, radiation, infections, injuries, exposure to toxic substances, and the use of chemotherapeutic drugs [5]. Semen volume, sperm motility, morphology and count are essential. In 2010, the World Health Organization (WHO) published a guide to report normal values [6]. It is termed normozoospermia if all semen analysis parameters are within normal range, oligozoospermia if sperm count is reduced (5-20 million /ml, severe: <5 million /ml) and azoospermia if there is no viable sperm in the semen. Azoospermia is divided into 3 types: Pretesticular, testicular and post testicular azoospermia [7]. Recent advances in assisted reproductive techniques (ART) have been a great hope for infertile couples to have a baby. The use of in vitro fertilization, which is one of the ART, can increase birth defects during fertilization and thus may lead to genetic or epigenetic abnormalities in the child. In addition, current ART has not been able to help infertile couples without functional gametes unless donor gametes are used. However, the development of stem cell technology may provide new therapeutic strategies for infertile couples [8,9] . In this review, we aimed to summarize the current studies on the role of therapeutic mesenchymal stem cells (MSCs) in the treatment of testicular damage.

Reasons of non-obstructive male testicular damage

Environmental Factors - Cigarette and alcohol

It is known that cigarettes contain a lot of toxic chemicals. Smoking leads to an increase in reactive oxygen species, thereby escalating oxidative stress, leading to DNA damage and apoptosis. Consequently, spermatogenesis, maturation of sperm, and sperm function all deteriorate, which may lead to infertility [10,11]. In addition, maternal smoking could be injurious on the fertility of the male offspring. Germ cell DNA damage and defective sperm production were detected in the male offspring with prenatal smoke exposure [12].

Excessive alcohol intake has a negative effect on the male reproductive system, because it decreases testosterone levels and increases FSH, LH and E2 levels, while impairing Sertoli and Leydig cell functions. It also deteriorates sperm motility and morphology, and reduces sperm concentration and count [13].

ROS and male infertility

Oxidative stress occurs when the defense of the antioxidant mechanism is inadequate in the face of an excessive increase in oxidants or reactive oxygen species (ROS) [14]. Excessive ROS impair sperm function and sperm morphology due to sperm membrane lipid peroxidation, and cause reduced motility and ineffective sperm-oocyte fusion [15,16]. When oxidative stress elevates, peroxidation of polyunsaturated fatty acids (PUFA) in sperm plasma membrane prevents the realization of normal fertilization in male germ line [14]. Lipid peroxidation disrupts membrane integrity, leading to enzyme inactivation, DNA structural damage and cell death. Oxidative stress not only affects the fluency of the sperm plasma membrane, but also the integrity of the DNA in the sperm nucleus and mitochondria. Oxidative damage causes DNA chain breaks, base degradation, DNA fragmentation, DNA lesion, and protein crosslinking. The rate of DNA fragmentation has been shown to increase in the ejaculate of infertile men. Changes in spermatogenic events lead to the elimination of abnormal immature sperm in the ejaculate. Immature sperm, on the other hand, leads to excessive ROS production and DNA damage. [17-20]. High levels of ROS disrupt the inner and outer membranes of the mitochondria. This leads to the release of cytochrome c from mitochondria, activating caspases and causes apoptosis [17].

Cancer Treatment and male infertility

The success of cancer treatment has increased with the combination of surgery, radiotherapy and chemotherapy. Radiotherapy and chemotherapy are gonadotoxic for testes in all age groups of men. In particular, the testicular germinal epithelium is highly sensitive to radiation damage with regards to total dose, fraction and treatment site. Radiotherapy can adversely affect the testes when targeted for malignancies in the retroperitoneum or pelvis before bone marrow or stem cell transplantation. Even low dose irradiation (such as 0.1 Gray) reduces spermatogenesis. Doses over 2 Gy result in oligospermia or azoospermia [21,22].

Chemotherapeutic drugs such as alkylating agents, procarbazine and cisplatin have a negative effect on spermatogenesis because they induce azoospermia and their effects persist for a long time after chemotherapy. Azoospermia occurs in treatments (such as Ewing Syndrome) using one or both alkylating agents, such as cyclophosphamide or ifosfamide. Furthermore, the combination of cisplatin with one of the alkylating agents causes oligospermia or azoospermia. Different combinations of chemotherapeutic agents used for the treatment of Hodgkin's disease also cause azoospermia. Although successful results are obtained with mechlorethamine, oncorin/vincristine, procarbazine, prednisone (MOPP / MVPP) in patients with hematopoietic malignancies, these chemotherapeutic drugs taken in childhood may cause azoospermia and hypogonadism in 85% of patients, and the effects may last up to 15 years [23-25].

Testicular torsion

Testicular torsion may reduce blood flow in the testis, thereby causing infertility in a 1/4000 male population younger than 25 years. Ischemia-reperfusion (I / R) injury occurs even if detorsion is performed as an emergency surgical intervention to prevent testicular damage. I / R injury can lead to infertility in men due to disrupted spermatogenesis and increased apoptosis of germ cells. The duration of testicular ischemia in torsion and the severity of twisted cord are important. When the twisted cord is intervened within 6 hours, the testicular damage is less, and if the severity of the twist is high, cell necrosis begins within 4 hours. Severe testicular atrophy is inevitable if a torsion of more than 360 degrees lasts more than 24 hours [26,27].

Mesenchymal Stem Cells (MSCs)

Multipotent stem cells self-replicate, differentiate and provide tissue integrity. These cells have the potential to renew themselves, maintaining homeostasis in tissues and organs. In addition, they proliferate throughout life and meet the number of cells required for the tissue. Mesenchymal stem cells have multipotent stem cells properties [28]. Mesenchymal stem cells are heterogeneous cells and it is not enough to identify only specific cell surface markers for the isolation of MSCs. Instead, the characters of MSCs, their differentiation into multiple cells and their phenotypic markers should be fully defined. In short, in order to evaluate the cells as MSCs, the cells must have plastic adherence and differentiate into various cells such as osteocytes, chondrocytes and adipocytes. Furthermore, the expression rate of cell surface markers such as CD105, CD73 and CD90 should be greater than 95%, and the expression rate of markers such as CD34, CD45, CD14, Cd11b, CD79, CD19 and HLA-DR should be less than 5% [29,30].

When MSCs are administered systemically to the body, they not only treat the damaged region by differentiation, but also affect other cells by inducing regulatory paracrine effects, such as stimulants and inhibitors. MSCs are determined to secrete various growth factors and cytokines such hepatocyte growth factor (HGF), insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor (VEGF) , platelet-derived growth factor, interferon gamma, fibroblast growth factor (FGF) and interleukin IL-6, IL-8, IL-10 [31,32].

Differentiation of MSCs to germ cells - *in vitro* experiments

Recent advances in stem cell technology have also brought a new perspective to treatment of infertility. Nowadays, infertility is no longer a desperate disease due to the studies on obtaining germ cells from cells of different origins [33]. It is thought that infertility will be treated by transplanting germ cells obtained from differentiated stem cells in vitro to gonads in patients of non-obstructive azoospermia [34].

Huang et al. [35] investigated whether human umbilical cord MSCs (HUCMSC) differentiate into germ cells. For this, the HUCMSCs in retinoic acid (RA), testosterone and testicular cell conditioned medium were incubated separately for 3, 7 and 14 days, respectively, and expressions of germ cell specific genes (Oct-4, Ckit, CD49, Stella, and Vasa) were determined with RT-PCR and immunocytochemistry [35]. RA is a polar molecule that is a derivative of vitamin A and binds to retinoid receptors in the spermatogonia nucleus, thereby increasing the expression of tyrosine kinase required for spermatogonia differentiation [36]. In this case, while the expression of Oct-4 gene was high in both control and differentiation medium-treated cells, expression of the other genes (except Vasa) was increased both on days 3 and 7 after incubation. Vasa expression was determined in 14 days by immunocytochemistry, and it was determined that HUMSCs could differentiate into germ cells [35]. In study of Ghasemzadeh-Hasankolaei et al. [37], the efficacy of transforming growth factor beta 1 (TGFβ1), BMP4 and BMP8 on the conversion of BM-MSCs to germ cells was investigated. These agents were administered to individual BM-MSCs for 21 days. Differentiation to germ cells was demonstrated by determining the expression of Germ cell specific markers by RT-PCR. After use of TGFβ1 in BM-MSC culture, spermatogonial stem cells (SSC) and spermatogonia-like cells were obtained, while BMP4 and BMP8b were shown to induce primordial germ cell formation in BM-MSCs. Thus, it was determined that TGFβ1 could play a prominent role in infertility and spermatogenesis [37]. In another study, RA and BMP4 were combined and applied to both BM-MSCs and adipose tissue-derived MSCs (AD-MSC) to compare their differentiation potency to germ cells by determining the expression levels of Mvh, Dazl, Stra8 and Scp3 germ cell specific genes. Although there was an increase in the expression of these genes in both BM-MSCs and AD-MSCs, the expression rate in BM-MSCs was higher than in AD-MSCs, indicating that the potential for BM-MSCs to differentiate into germ cells is stronger [33]. The properties of AD-MSCs such as antioxidants and immune modulators are known. The secretomes of these cells are therefore very valuable. Oxidative stress has a major role in the development of male infertility. Therefore, oxidative stress in human sperm was induced by H_2O_2 in a study. These cells were then incubated for 24, 48 and 72 hours with conditioned medium of AD-MSCs (ADMSC-CM) to investigate the effects of ADMSC-CM on sperm vacuolation, DNA fragmentation, and oxidative stress levels. Sperm vacuolization and DNA fragmentation decreased significantly in the samples incubated for 24 hours, while other parameters remained stable [38].

Application of MSC in testicular damage - in vivo experiments

In recent years, after in vitro studies, in vivo studies have been accelerated and MSC application studies on damaged testicular tissues have increased. For this reason, many researchers have investigated the differentiation potential of MSCs obtained from various sources to germ cells and whether MSCs settle in testis and ameliorate testicular damage (Table 1). According to these studies, MSCs from different sources can restore spermatogenesis and the testicular niche (Figure 1).

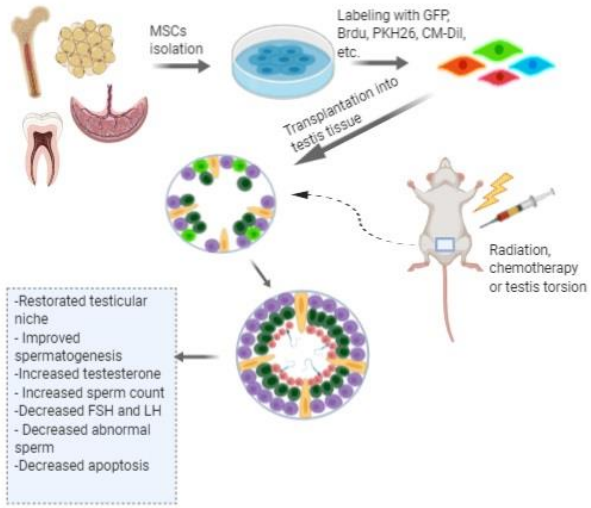

Figure 1: Mesenchymal stem cell isolation and transplantation into rat or mice with azoospermia. MSCs isolated from various sources such as bone marrow, adipose tissue, umbilical cord, placenta, and tooth. Isolated MSCs transplanted into testis (seminiferous tubules, rete testis or efferent duct) after labeling with Green fluorescent protein (GFP), Bromodeoxyuridine (Brdu), Paul Karl Horan 26 (PKH26), CM-Dil and etc. MSCs can restore spermatogenesis and testicular niche.

Table 1: Recent in vivo studies of MSCs application for male infertility

MSC Source	Number of cells	Used animals	Disease model	Period of MSCs Treatment	Results	Reference
Rat ADMSC	$1x10^6$ cells	Rat	Busulfan induced azoospermia	12 weeks	$GFP^+/Vasa^+$ and $GFP^+/SCP1^+$ cells were determined. Full spermatogenesis recovery and proliferation	[39]
Rat BMMSCs	$1x10^6$ cells	Rat	Lead (Pb) induced gonado-toxicity	21, 30 and 60 days	BMMSCs can differentiate into germ cells and Leydig cells. BMMSCs modulated testosterone levels and DNA apoptosis	[47]
Human UCMSC	$2,5x10^5$ cells	Mice	Busulfan induced infertility	3,9,18 and 20 days	HUCMSCs differentiated into germ cells and restored tubules	[40]
Induced BM-MSCs by co-culture with testicular cell conditioned medium	$1 x10^5$ cells	Rat	Busulfan induced azoospermia	8 weeks	BMMSCs can transdifferentiate into spermatogenic cells but after 8 weeks meiosis was not determined	[48]
Rat BMMSCs	$2,5x10^5$ cells	Rat	Busulfan induced infertility	4, 6 and 8 weeks	BMMSCs migrated to the germinal epithelium and expressed spermatogonia markers so these cells differentiated into spermatogonia	[49]
Human UCMSCs	$1 x10^5$ cells	BALB/c mice	Busulfan induced azoospermia	12 weeks	After transplantation of UCMSCs, increased expressions of meiosis-associated genes. UCMSCs (CD34-) restored testicular injury and decrease FSH and LH levels.	[41]
Rat BMMSCs	$5x10^6$ cells	Rat	Cadmium-induced testis injury	2 weeks	BMMSCs can prevent mitochondrial apoptosis and repair testis injury	[42]
Rat BMMSCs	$1x10^6$ cells	Rat	Doxorubicin-induced testicular toxicity	8 weeks	BMMSCs reduced rate of abnormal sperm and testicular oxidative stress	[44]
Human orbital fat tissues (OFSC)	$3x10^4$ cells	Rat	3 hours 720^0 torsion and detorsion	7 days	OFSCs can prevent intrinsic apoptosis and oxidative stress	[45]

Cakici et al [39] obtained ADMSC from rat adipose tissue and transplanted them into busulfan-induced azoospermic rats by labeling with GFP. The detection of GFP / Vasa and GFP / SCP1 positive cells showed the presence of ADMSCs in the tissue and these cells differentiated into spermatogenic cells after 12 weeks. Bromodeoxyuridine-labeled human UC-MSCs (HUMSCs) were transplanted in another azoospermia model using chemotherapeutic busulfan and detected in the tubule even after 120 days. It was determined that these cells settled, proliferated and differentiated into germ cells (via Oct-4, α6 integrin, C - kit, VASA expressions) [40]. A comparison study of the effects of stem cells on testicular injury was also conducted. Researchers isolated stem cells from umbilical cord origin. Umbilical cord blood-derived hematopoietic stem cells (HSC, CD34 + cells) and UCMSCs (CD 34-cells) were transplanted into mice with busulfan-induced azoospermia, and increased expression of meiosis-associated genes were determined in the MSC transplanted group (Vasa, SCP3 and PgK2). The MSC group (CD34-) restored testicular injury by increasing spermatogenic gene expression, whereas the CD34 + group showed no activity [41]. Cadmium is a heavy metal used in industrial and agricultural production and may cause infertility by reducing the number of sperm on people exposed to this toxic substance. Researchers transplanted 10^7 BM-MSC and studied whether BM-MSCs could restore cadmium-induced rat testicular damage and the role of mitochondrial apoptosis in this process. Expression of apoptosis-related proteins (Bim, Bax, Cytochrome C, Caspase-3, active Caspase-3 and AIF increased, Bcl-2 decreased) was determined 2 weeks after transplantation. Thus, it has been shown that mitochondrial apoptosis may be highly associated with BM-MSCs repairing damaged testicular tissue damage in rats [42].

Researchers are turning to different sources of MSC for the treatment of testicular injury. For example, Maghen et al [43] evaluated the first trimester human umbilical cord perivascular cells (FTM HUCPVCs), a new source of MSC. These cells have been shown to express and secrete factors known as important regulators of the testicular cell line such as FGF2, GDNF, LIF and BMP4. In addition, FTM HUCPVCs were found to have supportive properties for testicular regeneration in vitro and in vivo. After transplanting FTM HUCPVC to testicular injury models, induced by mono-2-ethylhexyl phthalate (MEHP), these cells were demonstrated to promote germ cell regeneration by the presence of Daz1 and acrosin positive cells. It has been reported that FTM HUCPVCs are capable of repairing the human testicular niche in case of testicular damage.

Chemotherapy causes irreversible damage and loss of fertility, especially in children and young men. For instance, although Doxorubicin (Dox) is an effective and widely used anti-cancer drug, its gonadotoxicity is quite high. Rats were injected intravenously with BM-MSC (2×10^6 cells) to investigate the effect of BM-MSCs against doxorubicin (Dox) induced toxicity in the assays. After 8 weeks of treatment, BM-MSCs reduced the high level of malondialdehyde caused by Dox and increased antioxidant levels to reduce testicular oxidative stress. Morphologically, testicular atrophy due to Dox, diameter of seminiferous tubules and germinative cell layer thickness were significantly reduced after BM-MSC transplantation. BM-MSCs were effective in restoring the structural efficiency of the reproductive system in testicular injury [44].

Hsiao and his team [45] transplanted 3×10^4 MSCs obtained from human orbital adipose tissue into 720^0 Torsion-Detorsion (T/D) rats by local injection into the testis. The effects of MSCs on oxidative stress and apoptosis mechanisms were investigated by superoxide dismutase 2, Bax, Caspase-3, P450, Sox-9 and malondialdehyde (MDA) test in T/D rats. MSCs were shown to reduce I/R-induced intrinsic apoptosis and oxidative stress. In addition, in their other study, they determined that T / D reduced sperm motility, sperm content, ATP content in sperm and F-actin expression, while MSCs injected to the testis increased ATP production by regulating glycolysis imbalance and Akt / GSK3 pathway. Sperm motility and energy were

increased with the help of MSCs in T/D induced-testicular damage thus, sperm function was repaired [45,46].

Conclusion

Male infertility is a fundamental problem and different treatments are investigated. Today, ART is widely used in couples who cannot have children. However, ART may be insufficient in some cases. For example, patients who received chemotherapy and / or radiotherapy in childhood may encounter azoospermia. Researches have focused on regenerative medicine and the development of stem cell technology has raised hope for infertility. MSCs from many sources have attracted the attention of researchers because of their high potential for both proliferation and differentiation. Although stem cells obtained from various sources are identified as MSC, their differentiation potentials to germ cells were different. Studies comparing the differentiation potentials of MSCs from different sources in testicular tissue are limited. Apparently, MSCs can improve testicular damage. In addition to proliferation in the damaged region, their paracrine properties can also improve the damaged tissue. MSCs secrete chemokines, cytokines, and growth factors, which may affect the testicular niche and restore the process of spermatogenesis. MSCs have brought a new perspective in the treatment of male infertility.

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