

Comparative Study of mesenchymal stem cells from bone marrow and adipose tissue in the treatment of cyclophosphamide induced ovarian damage in adult female albino rats

Mohamed Abdallah Ahmed Gebba, TALAAT MUSTAFA MOHAMAD, GAMAL ELSAYED ABD-ALSALAM, KAMAL MUSTAFA KAMAL, EMAN ALI ELBANNA.

The present study aimed to compare the therapeutic effects of mesenchymal stem cells (MSCs) derived from the bone marrow (BM) and adipose tissue (AT) of rats against toxic effects of cyclophosphamide (CLP) on the female gonads of experimental rats using histological analysis.

In this study we used fifty adult Albino rats (10 male & 40 female, average weight of 200-220 gm each). The recipient animals were kept under standard conditions, at 22–25 °C with a 12-hour light/dark cycle, with food and water freely available throughout the study.

1st In vitro study:

This experimental study was conducted on 10 adult male albino rats (6 weeks old) for isolation and culture of BM-MSCs & AT-MSCs followed by culture of separated MSCs.

2nd In vivo study :

Forty female rats were randomly divided into four groups. Rats in (***group I***) included 10 rats were divided into (***subgroup Ia***) (***negative control group***). It consists of 5 rats that were chosen randomly as (normal healthy rats), and not received any drugs, another ***subgroup Ib*** (***positive control group***) included 5 rats were injected with a single intra-peritoneal dose of 0.5 ml PBS/100 gm body weight ***Group II (CLP group)***: included 10 rats were injected with single dose of CLP intraperitoneal in a dose of 50 mg/kg-B.W of rats. Followed by a dose of 8 mg/kg every week on the eighth and fifteenth days, after 3 weeks, the rats were sacrificed to obtain their ovaries, ***Group III (IOF+BM)*** included 10 rats were injected with CLP as group III. After 3 weeks, these rats were injected with BM-MSCs intravenous through the tail veins in a single dose (5×10^6 cells/ml) in a -132-

Summary and Conclusion

volume of 0.3 ml of 0.1mol/l ml PBS (pH 7.4). The rats will be sacrificed after 30 days from cell therapy. ***Group IV (IOF+AT)*** included 10 rats were injected with cyclophosphamide as group III. After 3 weeks, these rats were injected with AT-MSCs intravenous through the tail veins in a single dose (5×10^6 cells/ml) in a volume of 0.3 ml of 0.1mol/l ml PBS (pH 7.4). The rats will be sacrificed after 30 days from cell therapy. The rats were sacrificed and ovaries were dissected, examined and prepared for light microscopic using Hx. & E., PCNA, CD44

immunostaining and morphometric studies.

We assessed the effect of transplanted cells on oogenesis via microscopic examination of ovaries.

Stem cell engraftment was detected using fluorescent microscopic (Leica) examination; PKH26-stained ovaries tissue emitted pink fluorescence that indicated homing of stem cells into the ovarian cortex. The present work demonstrated that Hx. & E. stained sections of control rat's ovaries showed normal morphology. It was covered by thin connective tissue capsule. The different types of ovarian follicles (primordial, primary, secondary and Graffian follicles) were densely packed and lined with multiple layers of granulosa cells with little interstitium-containing clusters of interstitial cells and blood vessels. In CLP treated group, CLP produced variable histological changes in the ovaries of the rats. These changes appeared as loss of normal architecture of the ovarian follicles, markedly reduction in the all types of granulosa cells which caused shrinkage of the follicles and subsequently dilatation of intercellular spaces, detachment of granulosa cells from the irregular and thickened basal lamina. Dilated congested blood vessels and vacuoles in the interstitial tissues of medulla were seen. CLP group also

-133-

Summary and Conclusion

showed decreased expression of PCNA. *BM-MSCs and AT-MSC treated rats showed* restoration of normal architecture. In this study it was observed that ovaries of the rats of group III & IV showed marked histological changes as compared to those of cyclophosphamide treated group II.

AT-MSCs treated group also showed relative improvement of normal architecture with minimal histological changes as compared to those of group III. AT-MSCs group showed increased expression of PCNA. BM-MSCs & AT-MSC treated ovarian tissue have shown positive cell membranous reaction for CD 44 in the form of brown pigmentation of cell membrane and cytoplasm of granulosa cells which means that transplanted BM-MSCs & AT-MSCs have differentiated into these granulosa cells as CD 44 characterizes MSCs.

-