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Can Transplantation Of Mesenchymal Stem Cells (MSCs) Provide Protection For Radiation Induced Mucosal Injury? (A Systematic Review And Meta-Analysis On Animal Studies.)

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Abstract: Objective: Patients who undergo radiotherapy may develop acute and/or chronic side effects resulting from gastrointestinal tract (GIT) alterations. In this study, we address the question of the regenerative capability of mesenchymal stem cells (MSC) after radiation-induced GIT mucosal injury.Method:we systematically assessed the evidence in the scientific literature for the effectiveness of MSCs in animal models of radiation mucositis regarding epithelial thickness preservation and proliferative /apoptotic activity of the tissue. SYRCLE's tool for assessing risk of bias was used to assess the internal validity of the included studies. **Data extraction and data synthesis**: Seven studies were included. Data regarding the animal model, intervention and outcome was extracted and tabulated. The quality of the studies was generally low regarding randomization, allocation and blinding. The heterogeneity was very high due to variability of animal model, intervention used, types of cells, dose and outcome assessment technique and timing. Epithelial thickness within first 2 weeks after irradiation was reported in 4 studies included in a meta-analysis. **Results:** Within first week results showed that the pooled effect estimate was no significant(MD 125.5 [-32.3, 283.3] P=0.12). Sensitivity analysis after excluding study measure the outcome after 3 days showed significant pooled effect estimate (MD 191.3 [143.06-239.5] P<0.0001).While within the 2nd week there was no significant effect estimate MD 36.6. [-16.8-89.73] P=0.18) and heterogeneity was very high (I^2=96%).Conclusions: Systemic injection of MSCs after irradiation decreasing the effect of radiation on epithelial thickness. However, this effect is significant in second week after irradiation. Further powered preclinical studies are needed considering less potential risk of different sources of bias before shifting for clinical trials.
Keywords: MSCs, Radiation mucositis, Animal studies, Meta analysis, Epithelial thickness.

Introduction

One hundred percent of patients receiving high dose of radiation therapy for head and neck cancers suffer from different degrees ofmucositis[1,2].Mucositis is an inflammation affecting the mucous membranes that line the whole length of the digestive tract causing atrophy, erythema, ulceration, and, eventually, the loss of mucosal barrier functions. This loss of function resulted from impairment of rapidly dividing mucosal cells that are responsible for the regenerative capacity of the oral and alimentary epithelium[3,4].

However, most of the current treatment modalities of mucositis are only palliative, neither specific nor efficient in preventing or treating such complication[5,6]**.**Therefore, more effective approaches for prophylaxis and treatment of mucositis are urgently needed. Many studies have shown that mesenchymal stem cells (MSCs) participate in the regeneration and repair of a variety of diseased epithelial tissues, including injured epithelial layers in skin[7]**,** gastric mucosa and intestine[8]**.**

To date, there are no clinical trials that support this preventive intervention. Nearly all available studies reporting the efficiency of the MSCs in management of radiation mucositis were animal studies, this rationalize the need for more animal researches to gain enough data that encourages clinicians to pass through clinical trials. Furthermore, high quality systematic reviews are essentially needed in order to analyze pre-clinical trials to be able to initiate a clinical one. Therefore, we conducted a systematic review with meta-analysis to have a closer view at the relation between animal studies on MSCs and radiation mucosal lesion.

Materials and Methods

1. Search Strategy and Selection of the Papers

Pub med and Scopus electronic databases were searchedfor original articles concerning the effects of MSCs on experimental radiation induced mucositis until September, 2016, in addition to Google scholar. Furthermore, the reference lists of the selected relevant papers were screened by hand for potentially relevant new papers. The search strategy was composed of two elements: MSCs and radiation induced mucositis. To detect all animal studies in Pub Med search filters were used. No language or date restriction was used.

2-Inclusion and exclusion criteria

The selection of studies was performed on the basis of the title and abstract. Two review authors (B.E, NY)independently screened all the abstracts for the inclusion criteria. Disagreements were resolved by a third methodological expert (R.S). Studies were included if they investigated the effects of MSCs on epithelial thickness, or proliferation, or apoptotic markers expressed in the mucosal tissues, and conducted on experimental model of radiation mucositis. No language or date restriction. Papers were excluded if they fulfilled one of the following criteria: (1) Not primary study (e.g. review or letter etc.); (2)Radiation injury affects tissues other than mucosal tissues. Data of interest was extracted and tabulated this includes characteristics of animal model (age weight, strain, sample size) Table 1, method of induction of radiation damage, type and dose of radiation, type of mesenchymal cells, dose of cells, timing of scarification, route of administration, (Table2) outcome measures epithelial thickness in micro-millimeter as a primary outcome, degree of proliferation and/ or apoptosis and type of marker used (Table 4). If data were only presented graphically, data were measured using universal on-screen digitizer whenever possible (Web Plot Digitizer version 3.10). With this software, it is possible to measure distances, areas and perimeters of figures on a computer screen.

3. Assessment of Risk of Bias in Included Studies

We assessed the risk of bias of the included studies using the criteria/items described in (Table 4) describing SYRCLE's tool for assessing risk of bias[9]. The criteria were independently assessed by two reviewer (N.Y) and (R.S) by using collectively predefined judging criteria. The score ''yes'' indicates low risk of bias, the score ''no'' indicates high risk of bias, ''unclear'' indicates unclear risk of bias.

4. Data Synthesis and Statistical Analysis or tables

For the outcome measure ''epithelial thickness, we extracted mean,SD, and number of animal in each group. This outcome was measured repeatedly on different time points, in the meta analysis we used data measured within 1 and 2 weeks to measure the early effect of the MSCs as we target prevention. However, for other outcome measures (i.e. Proliferation and apoptosis), data were discussed and presented in the tables but not included in meta-analysis. The software used to perform meta- analysis was (**RevMan 5 version: 5.3.5).**

Results

1-Description of the Included Studies

The search strategy retrieved 25 papers in PubMed and 3 papers in Scopus 45 papers in Google scholar. Initially, 12papers seemed to meet our selection criteria. After studying the full-text articles, 7 original studies remained[10,11,12,13,14,15,16]as 2 excluded for being reviews[17,18]and 3 as they did not report any of the interested outcomes in our review[19,20,21]*(Fig 1)*.The characteristics of these studies are shown in (Table 2, 3, 4). The study characteristics varied considerably between the included papers. Six studies were performed with mice and one used rats. Four studies used only males, one study used females, and one paper did not mention the gender of the animals' weight and ages of the animal also varied considerably Table 2. Different radiation sources and doses were used to induce radiation mucositis. Also the doses of MSCs, the route of administration, the scarification dates and time for assessment varied greatly between studies Table 3**.**

2-Risk of Bias and Quality of Reporting

Table 4 shows the overall results of the risk of bias assessment ofthe 7 studies included in this review[10,11,12,13,14,15,16]**.**In (14%) of the studies, the allocation of the experimental units to the treatment groups was randomized. None of the papers described whether or not the allocation to the different groups during the randomization process was concealed. Also, none of the studies reported that they blinded the outcome assessment. However, all the studies showed low risk of performance bias as the different groups treated equal and in the same time.

3 - Effects of MSCs

3.1. Epithelial thickness

Four experiments studied the effect of MSCs on epithelial thickness in experimental radiation mucositis**[11,12,14, 15, 16]**. Effect was assessed within first week after irradiation in all the studies (all included in the meta analysis (comparison 1). While, three of these studies could be included in the meta-analysis within 2nd week after radiation (comparison 2) as in one study [14]**(**Data at this time point was not shown).

In (comparison 1) two of the four included studies[13,14]showed a significant increase of epithelial thickness in MSCs group versus irradiated only group. While, in meta- analysis there was no significant pooled effect estimate (Fig 2: MD 125.5 [-32.3, 283.3] P=0.12). Heterogeneity was high (I^2=100%).

In (comparison 2) within 2 weeks two of the 4 included studies[12,13]showed a significant increase of epithelial thickness in MSCs group versus irradiated only group. In the meta- analysis there was also a significant pooled effect estimate (Fig 4: MD 75.56 [12.25, 138.87] P=0.02). However, heterogeneity was high(I^2=98%).

3.2. Proliferation

Three out of seven of the included articles [10,13,14]investigated the effect of MSCs in experimental model of radiation mucosal injury on proliferation of the cells. However, they could not be included in meta-analysis. Immunohistochemical method was used in all the studies to measure the expression of different proliferation markers in tissues by different analyzing techniques in different time points.

In Aboushady et al. [10] the tissue PCNA marker was detected at 15 days after radiation The immunostained sections were examined using an image analyzer computer system to assess the optical density of the immunostain, the results showed that the mean value of PCNA, in the treated group (mean ± SD = 76.13 ± 4.38), was significantly increased than that of the irradiated group (mean ± SD = 54.24 ± 11.71).

In Semont et al.,[15] the number of proliferating crypt cells was assessed at 3 days on histological slides of small intestine stained by Ki67 antibody and the proliferation index(number of Ki67-positive cells per five crypts) was used as a measure. Results showed that, after human MSC infusion into irradiated mice, the crypt cell proliferation index (peaks at a value of (mean ± SD 206.3±9.9) increased by 61.0% versus irradiated animals (mean ± SD 130.08 ±20.1In Saha et al.[13] the percentage of the BrdU+ve crypt epithelial cells synthesizing DNA was significantly enhanced in MSCs group at 3.5 days post- irradiation (mean±SD 42.82) versus irradiated only group (mean±SD 23.43).

3.3Apoptosis:

Semont et al. [15] is the only study investigated apoptosis, apoptotic cells were studied at 3 days on histological slides of small intestine by TUNEL assay (percentage of TUNEL-positive crypt–villus). Results showed that thehMSC + irradiation group (3 days) the percentage of crypt–villus axis containing apoptotic cells decreased significantly than irradiated only group (reduction of 51.3% versus irradiated animals). Fifteen days after irradiation and hMSC infusion, the number of apoptotic cells in crypt compartments fell back to control values (data not shown in the paper). Table 4.

Table 1: Animal characteristics

Study	*No of animals in each study groups*	*No of animal in control group*	*Sex*	*Strain name*	*weight*	*Age*
(Aboushady et al .2012)	10	10	male	Albino rat	100-150 gm	10 w
(Se´mont et al.,2010)	N/A	N/A	male	NOD/SCID mice	200-250 gm	10 -12 w
(Goa et al .2012)	N/A	N/A	male	C57BI/6 (NCI-Fort Dietrich, M D), mice	N/A	Five- to 6-weeks-
Osama et al. 2016	3-5	3-5	N/A	BALB/c mice	N/A	N/A
(Zhang et al .2008)	10	10	N/A	b-Gal-transgenic mice that constitutively express the LacZgene (B6.129S7-Gtrosa26)	N/A	N/A
(Sémont et al .2006)				immunotolerent NOD/SCID mice		
(Gaoet al .2012)	10	10	male	BALB/C mice	n/a	10 -12 w

Table 2:Data about intervention and model induction

study	*Irradiation*			*Treatment with MSCs*				*Time of sacrificing the animal in days*
	dose	*site*	*type*	*Source of cells*	*Route of administration*	*Dose*	*Groups*	
(Aboushady et al .2012)	10 Gy	Head &neck	Cobalt 60 source	BM-MSCS Allogenic (other rats)	Local injection	(1.0 × 107 cells in 0.2 ml)	control group (G1) Irradiated only group (G2) Irradiated+MSCs	15 days after irradiation
(Se´mont et al.,2010)	a total dose of 8.5 Gy to the abdominal region	Total body and abdominal region	n/a	human BM-MSCs	Intravenous injection via tail vein	5×106 hMSC	control group (G1) Irradiated only group (G2)]Irradiated+Cs group (G3)	3, 15, 30, 60, 90, 120 days after irradiation

(Osama et al .2016)	18GY	Head and neck region	n/a	adipose tissue–derived MSCs (aMSCs)	Interperitoneal	5 doses of 2.5 million freshly cultured syngenica MSCs	Irradiated only group Irradiated+aMSCs group.frozen Irradiated+aMSCs fresh group. Irradiated+fibroblast group.	8,9.10.14 days after irradiation
(Zhang et al .2008)	13Gy	abdominal region	Cobalt 60 source	Bone marrow MSCs were isolated from 6-week-old b-Gal-transgenic mice	Intravenous injection via tail vein	(1.0 × 107 cells in 0.2 ml).	control group (G1) Irradiated and salinegroup (G2) Irradiated+ NULL MSCs group (G3). Irradiated+ Ad-mCXCR4MSCs group (G4).	At 5 and 10 days after irradiation
(Gaoet al .2012)	of 10 Gy	abdominal irradiation	((60Cobalt-	Human umbilical cord (UC)-derived MSC and high molecular weight fraction (HMWF) from hypoxic-conditioned media of UC MSC	Intravenous injection via tail vein	10 6 MSC/200 m L/PBS	Control group Radiation +high molecular weight fraction for 7days(HMWF-7) Radiation +HMWF-1(for once) Radiation +MSCs	after30 days

(Saha et al.2011)	16 GY	abdominal irradiation	AIR	Bone marrow MSCs	Intravenous injection via tail vein	(2×106 cells/mice	control Radiation Radiation +MSCS Radiation +MSCs fraction CD11b+ve	15 days
(Se´mont et al.,2006	a total dose of 8.5 Gy to the abdominal region	Total body and abdominal region	n/a	human BM-MSCs	Intravenous injection via tail vein	(5×106 cells/mice	Control AI+ MSC AI only MSC only	3-15 days

Table 3: Outcomes' measurement (proliferation, apoptosis, thickness)in irradiated only(control) and MSCs groups

	study/ groups	***epithelial proliferation***		***epithelial apoptosis***		***epithelial thickness in µm (1st week)***	***epithelial thickness in µm (2nd week)***
		Marker	***Measurement***	***Marker***	***Measurement***	-	
Aboushedy et al., 2012	***Control Group***	***PCNA***	***54.24±11.7***	***-----***	***--------***	***-------***	---------
	MSCs Group	***PCNA***	***76.13±4.38***	***------***	***--------***	***----------***	-------
Semont et al., 2010	***Control Group***	***ki 67***	***150.66±6.7***	***TUNEL***	***87.7±2.2***	***280±10***	---------
	MSCs Group	***ki 67***	***206.3±9.9***	***TUNEL***	***57.4±2.1***	***530±5.2***	----------
Zhang et al., 2008	***Control Group***	----------	***---------***	***----------***	***--------***	***189±4.20***	*235.3±6.5*
	MSCs Group	***-------***	***---------***	***----------***	***----------***	***196.5±4.6***	*249.5±7.2*
Gao et al., 2012	***Control Group***	***----------***	***---------***	***---------***	***----------***	***363±61***	***438.6 ±60***
	MSCs Group	***-------***	***--------***	***-------***	***---------***	***385±63***	***444.8±48***
Saha et al., 2011	***Control Group***	**BrdU**	***23.43±2.01***	***--------***	***---------***	***--------***	***-----***
	MSCs Group	**BrdU**	***42.82±1.66***	***---------***	***-------***	***----------***	***----------***

Semont et al., 2006	***Control Group***	-------	-------	--------	------	***400±11.5***	*346.7±14.6*
	MSCs Group	-------	------	---------	--------	***180±17.5***	*425.4±11.4*
Osama et al 2016	***Control Group***	------	----------	----------	-------	--------	***10.7±13.9***
	MSCs Group	--------	---------	----------	------	---------	*210.8±34.8*

Table 4: Risk of bias assessment according to SYRCLE's tool for assessing risk of bias

Question	**Aboushady *et al., 2012***	***Gao et al., 2012***	**Osama et *al., 2016***	***saha et al., 2011***	***semont et al., 2006***	***semont et al., 2010***	***zhang et al., 2008***
1-was it stated in the method section that the experiment was randomized	no	no	no	no	no	no	yes
2-was the method of randomization adequate	no	no	no	no	no	no	unclear
3-were the groups similar at baseline	unclear	unclear	unclear	unclear	unclear	unclear	unclear
4-were the caregivers blinded for the allocation of the animals to the specific groups	unclear	unclear	unclear	unclear	unclear	unclear	unclear
5-was the outcome assessment blinded	unclear	unclear	unclear	unclear	unclear	unclear	unclear
6-methods for outcome assessment the same in both groups	yes	yes	yes	yes	yes	yes	yes
7-is the timing of the intervention during the day similar in both groups	yes	yes	yes	yes	yes	yes	yes
8-was the outcome assessment randomized across the groups	unclear	unclear	unclear	unclear	unclear	unclear	unclear
9-number of excluded animals specified per experimental group for each outcome measure	yes	yes	yes	yes	unclear	yes	unclear
10-reason for exclusion mentioned for each excluded animal	unclear	unclear	unclear	unclear	no	no	no

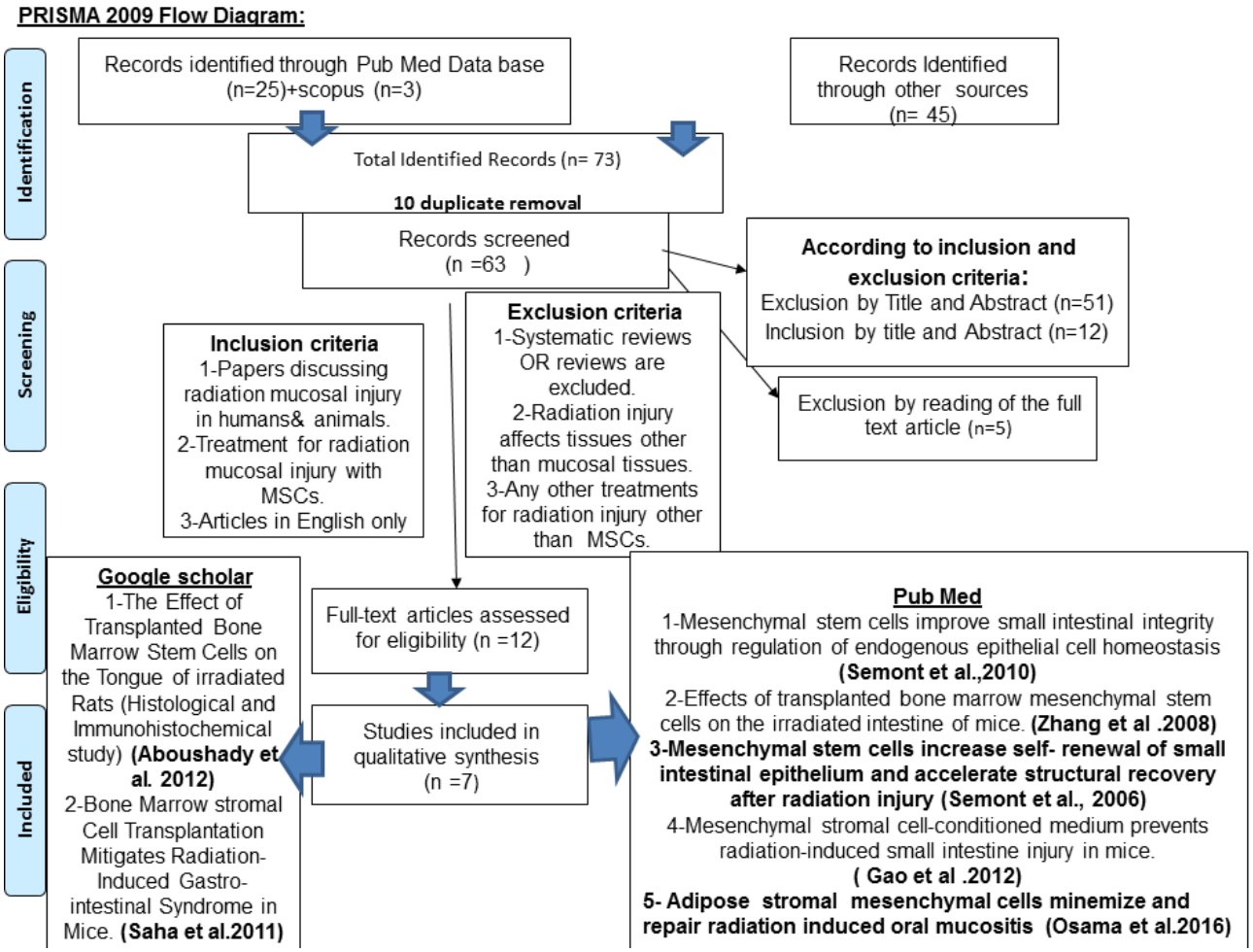

Fig.1: Prisma flaw chart

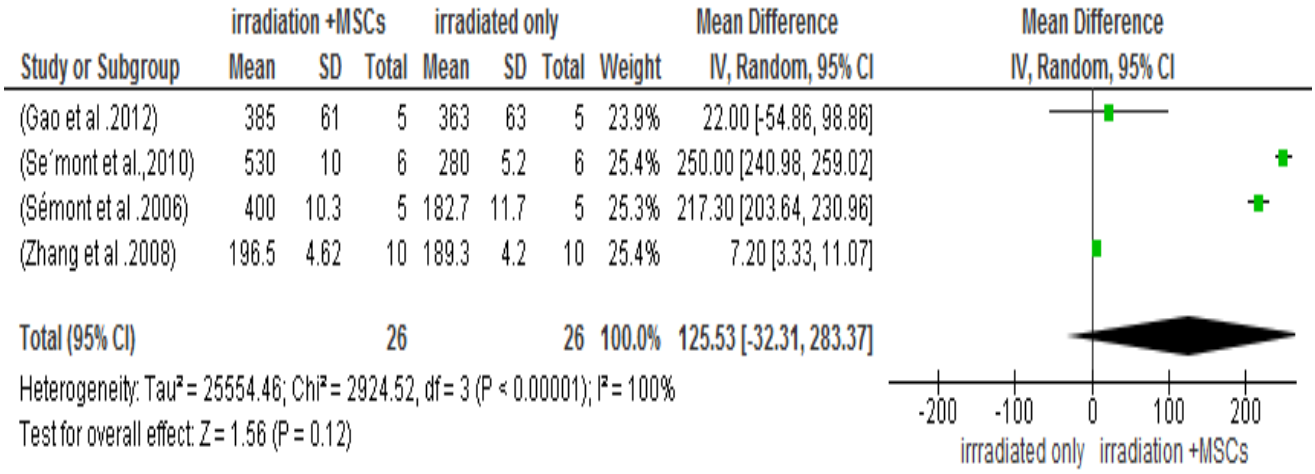

Fig.2. Forest plot showing results of meta-analysis comparison 1 epithelial thickness in MSCs versus irradiated only within 1st week

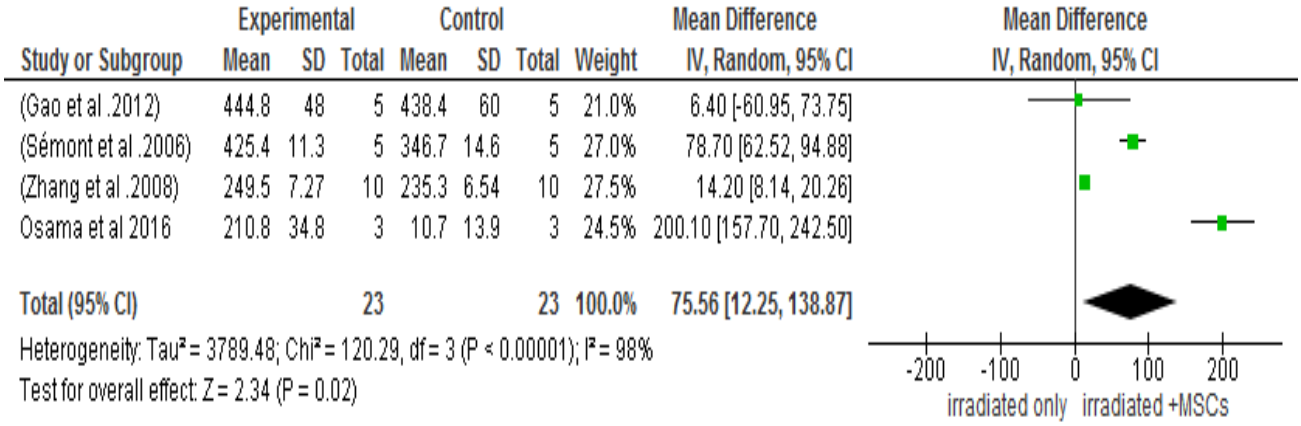

Fig.3 Forest plot showing results of meta-analysis comparison 2 epithelial thickness in MSCs group versus irradiated only group within 2nd week after radiation

Discussion

Seven animal studies were included in this systematic review of evidence evaluating the role of MSCs in ameliorating the effect of regional radiation on normal epithelial thickness and epithelial homeostasis (proliferation and apoptosis). There was a considerable heterogeneity in studies characteristics, outcome assessment methods and timing of assessment between the included studies which was an obstacle in pooling studies that reportsproliferative activities into statistical analysis.

For apoptotic activity only one study reported this outcome .On the other hand, for epithelial thickness five studies reported this outcome but in different time points. As our concern is about the preventive role of the intervention, changes within first 2 weeks after radiation was included in the meta-analyses. We used random effect model as we expected considerable heterogeneity. Upon evaluation of results, we found that there is overall increase of epithelial thickness and proliferative activity in MSCs group in the first week but this effect on epithelial thickness was not significant while, it was significant during the 2nd week. In addition, reduction of the apoptotic activity was reported in only one study at 3 days[11].

Uncertainty about the quality of the available evidence is mainly attributed to shortage of high quality studies, small sample size, methodological limitations and increased risk of biases and inconsistency across studies. Inconsistency refers to the dissimilarity of estimates of effect across studies.

Several in vivo and in-vitro studies were previously conducted to detect the role of MSCs in modifying the rate of cellular growth and/or apoptosis. Higher cellular proliferation with obvious reduction of apoptotic rate of renal epithelial [22]and nerve cells [23]were clearly detected in vivo as a result of MSCs therapy. While other in vitro studies have showed similar effects on the renal epithelial cells[24], endothelial cells [25], and cardiac cells [26].

Recent other preclinical studies evaluated the effect of the MSCs on the severity and the duration of radiation induced oral mucositis models [12,19,20], they did not investigate the proliferation or apoptosis but evaluated different aspects related to clinical endpoints .

In2014 a study by Schmidt and his group was conducted on the effect of MSCs therapy for radiation induced oral mucositis (RIOM) they concluded that transplantation of bone marrow (BM) or BM-derived MSCs (BM-MSCs) could modulate RIOM in fractionated radiotherapy in mouse model depending on the time of transplantation relative to radiation exposure time

However, Schmidt et al.**[20]** in his single dose radiation mucositis model defined the potential of mobilization of endogenous bone marrow (BM) stem cells by rHuG-CSF or mobilization of bone marrow transplantation (BMT) to reduce the effect of single-dose irradiation on mouse oral epithelium and they assessed the ED50 (dose at which ulceration is expected in 50 % of the animals).

Results showed that the response of oral mucosal epithelium to a single radiation exposure can be significantly reduced by post-exposure mobilization, but not by transplantation, of BM stem cells.

In Osama et al.[12] study, they investigated the ability of freshlycultured adipose tissue MSCs therapy given intrapretonialto minimize and/or repair the single dose radiation induced oral mucositismouse model. Results showed that aMSCs significantly minimized and repaired radiation-induced oral mucositis with a 72%

reduction in ulcer duration. Adipose tissue derived MSCs (aMSCs) dose size and frequency, number of doses and therapy onset time are the main keys for optimized therapeutic outcome. .

In MSC-based therapy for repairing the lesions associated with radiation mucosal injury in alimentary canal, especially for using heterogenic MSCs, the mechanism of actionis mainly attributed to the autocrine/paracrine actions achieved by MSCs. According to recent advances, several putative actions of radiation enteropathy management achieved by MSCs. The suggested putative actions by which MSCs repair radiation mucosal lesions could be explained primarily as; the engrafted MSCs induce infiltrated immune cells to switch from pro-inflammatory to anti- inflammatory cytokine secretion thus, enhance anti-inflammatory events. As a secondary effect, repair responses are enhanced by systemic events, such as elevated levels of regenerative initiators, despite the rapid disappearance of donor MSCs[27].Thus, cytokine accelerate regeneration of the injured tissue as reviewed in [28].

Analyzing the main studies characteristics, mice is considered the model of choice in all the studies and intestinal mucosa was tissue of interest except [10]who used rats due to convenience and easy manipulation as he studied oral mucosal tissues. Regarding type of MSCs, all studies used bone marrow derived mesenchymal stem cells (BM-MSCs) except Gaoet al.[14]who used human umbilical cord blood cells and Osama et al. [12]who used adipose tissue derived MSCs.

Several researches have discussed the unique character of BM-MSCs which is called plasticity. This property makes such cells able to differentiate to mature cells of various tissue types[29] as mucosal cells of GIT [30].Furthermore, such differentiation power could be preserved during the expansion process in a culture media containing 20-30 population doublings[31].

Regarding MSCs, they are promising for cellular therapies because of their prominent anti-inflammatory effects, enhancing interleukin (IL)-10 secretion, ease of isolation, high cell count after expansion and their source abundance [32]. In radiation-induced normal tissue injury, aMSCs have shown significant repair of RT-induced cutaneous syndrome[33,34,35,36]. In addition, aMSCs found to be relatively resistant to ionizing radiation, a property that qualifies them to be a reliable cellular therapy candidate before and during RT [12].

Regarding route of administration all the included studies used intravenous injection exceptAboushady et al.[10]who injected the cells locally and Osamaet al.[12] who used intraperitoneal route. Despite, the advantages and efficiency of using the minimally invasive intravascular injection, the obligatory passage of the injected cells through lungs may cause entrapment of such cells due to its diameter(20-30 μm)[37,38]. Experiments with IV-delivered MSCs in a mouse model require the use of at least 1 × 106 cells and, more frequently, a dose as high as 5 × 106 cells/mouse to observe any effect[39]. This explain the high absolute numbers of cells used to ensure that a minimum number of cells reach the injury site distal to the lungs in the included studies.The intraperitoneal route allows to give higher dose size and volume. In addition, avoiding the local transplantation route, which could lead to added local injury, suffocation and animal loss due tovolume and mass effect after transplantation [12].

To summarize, the effect of the MSCs therapy in protection from radiation injury is still under investigation. Researches concerning this point, although promising,yet face many difficulties. Obviously, the heterogeneity of these studies emphasizes that there is stillno definite outline or protocol for treatment regarding the source of cells, the dose,frequency, severity of the injury, route of administration, timing of the treatment relative to radiation exposure, model of the lesion and assessment of the effect. In general from our data we can conclude that systemic use is better than local delivery of the cells especially the interperitoneal route, the effect on epithelial integrity is not significant during first week and appeared to be significant during the 2nd week. The BM- MSCs is the most commonly used cell type also, aMSCs showed promising results.

Limitation of this work

The outcomes of choice were the outcomes testing the theory behind using the MSCs. Thus, were mainly histologic no clinical or functional analysis included as they are variable and could not give us over all estimate of the effect.

Research implication

The results of our work could draw the route for further research in the field by high lightening the main pitfall in the previous preclinical studies, sample size calculation is highly recommended. Special attention should be given in reporting animal studies, deficient data hinder assessing the internal validity of the studies.

Conclusion

According to our findings in the present review, systemic injection of MSCs after irradiation decreases the effect of radiation on epithelial thickness by maintaining cellular and tissue homeostasis (increase proliferation and decrease apoptosis). However, this effect is begun to be significant during

2^{nd}week after irradiation. Further powered preclinical studies are needed with consideration to decrease potential risk of different sources of bias before shifting for clinical trials.

Disclosure

This paper's contents are solely the responsibility of the authors

Competing Interests

None of the authors have any competing interests with respect to this paper.

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