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ORIGINAL ARTICLE

Stem cells improve the quality of colonic anastomoses - A systematic review

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Summary

Purpose: *Stem cells have multiple ways of differentiating and restoring healing. This feature may recommend their usage for decreasing the incidence of anastomotic fistulas in the colon in case of colorectal malignancy.*

Methods: *To determine whether stem cells are improving digestive healing, we performed a literature review using as Mesh terms: "anastomotic leak", "stem cells", and "colonic anastomoses", followed by an observational analysis on 3 experimental studies.*

Results: *We found that stem cells increase bursting pressure by an elevated rate of angiogenesis. In addition, the hydroxyproline content of the anastomoses is significantly increased in the stem cell group. The results concerning microscopic characteristics of digestive healing varied markedly between studies.*

Conclusions: *These findings suggest a novel role for mesenchymal stem cells in digestive sutures on ischemic conditions. Although stem cells have shown their beneficial effect on anastomotic healing, further studies are necessary to establish the indications, the appropriate method of administration, the sampling site and the identification of substances whose combination might potentiate their angiogenic effect.*

Key words: *anastomosis, angiogenesis, colon, leakage, stem cells*

Introduction

Digestive fistulas are an important complication of colorectal surgery in case of neoplasms, which significantly affect the length of hospital stay, the risks of nosocomial infections, and the costs, while altering prognosis and increasing the postoperative mortality rate by 8 to 10 times, which is considered to have forensic implications [1–3].

Despite progress in intensive care and new techniques for achieving digestive anastomoses, fistula occurrence rate is reported to range between 1.5 and 16% [4–6].

Knowledge of the risk factors has led to the development of several methods for reducing the incidence of anastomotic dehiscence either by control of the local factors or by correction of the general factors.

The new directions in the study of reducing digestive fistula incidence are turning to the use of stem cells in achieving a good anastomosis either by direct injection in the suture trenches or by suture loading. Stem cells can be derived from the bone marrow or from the adipose tissue, and are involved in the healing process by increasing angiogenesis, local blood flow, the activity of fibroblasts and collagen synthesis [7].

However, further studies on the use of stem cells to reduce the incidence of anastomotic dehiscence are needed in order to establish the indications, the mode of administration and the associated factors that may potentiate their effects and long-term prognosis.

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Table 1. Search strategy (Pubmed, Cochrane, Embase)

Number of search	*Term*
1	"anastomotic leak"
2	"stem cell"
3	"colonic anastomoses"
4	2 and 3
5	4 and 1

In this article we performed a systematic literature review to assess the benefits of stem cell use on the healing of colonic anastomoses.

Methods

Protocol and registration

Methods of the analysis and inclusion criteria were specified in advance and documented in a protocol (Table 1).

Eligibility criteria

Our study included only experimental research carried out on rat type models that had received stem cells harvested either from the bone marrow or from the adipose tissue. The studies performed monitored colonic anastomoses healing.

We reviewed papers published in English since 2000. No publication status restrictions were imposed. Studies were identified by searching electronic databases and scanning reference lists. This search was applied to Medline, Embase, and Cochrane (2000-present). The last search was run on 1 September 2014. We used the following search terms to find all studies and databases: "anastomotic leak", "stem cell", "colonic anastomoses" (Table 1).

Study selection

Eligibility assessment was performed independently in an unblinded standardized manner by 2 reviewers. Disagreements between reviewers were solved by consensus. All titles and abstracts were scanned and appropriate citations were reviewed.

Reviewers extracted information from each included study, using a special standardized form. No further contact with the authors was necessary. We extracted data on: (1) the source of the stem cells, (2) the mode of administration, (3) the concentration of stem cells, (4) the period after which healing was assessed (5) the healing parameters taken into consideration, (6) the frequency of the anastomotic fistulas.

Summary measures

The primary outcome was the mean difference between bursting pressure in the 2 study groups: with and without stem cells.

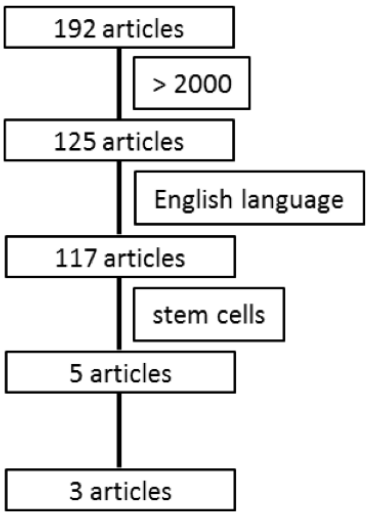

Figure 1. Flow diagram.

Results

Study selection

Three studies were identified for inclusion in the review. A hundred and ninety-two articles were identified when searching in the Medline, Embase and the Cochrane Library. Out of these, 117 papers were selected based on the predefined filters (articles published after 2000 in English) and reviewed independently, based on the content of the abstract. Of these, only 5 referred to the use of stem cells. After exploring their content the reviewers excluded 2 more articles (Figure 1).

Study characteristics

Methods

All three studies used multipotent stem cells harvested either from the adipose tissue or from the bone marrow. The collected stem cells were obtained by successive passages on culture media and used only after they demonstrated ability to differentiate.

On similar weight rats the authors performed single layer end-to-end colonic anastomoses. The maximum period of follow up of the subjects was 21 days. As Table 2 shows, the size of the groups differed [7–9].

Outcomes

In all studies the primary outcome was burst-

Table 2. Characteristics of the studies

Study	*Stem cell origin*	*Group number (n)*	*Animal weight (grams)*	*Anaesthesia*	*Model*	*Parameters monitored*	*Follow-up period (days)*	*Mode of administration*
Pascual 2008 [8]	Adipose tissue	5	250-360	Ketamin+ Atropine + Diazepam	-	fistulas expansion stenosis obstruction adhesions bursting pressure	4, 7, 14, 21	Biosutures $1.5*10^6$
Adas 2011 [9]	Bone marrow	10	250-280	Ketamin	Ischemic	bursting pressure hydroxyproline histo-pathological features	4, 7	Perianastomotic injection 10^6
Joo 2012 [18]	Adipose tissue	30	370-450	Ketamin+ Isofluran	Ischemic	body weight adhesions bursting pressure strictures histo-pathological features	7	Perianastomotic injection 10^6

Table 3. The effect of stem cells on the incidence of anastomotic fistulas on day 7 after surgery

Study	*Control group*	*Stem cell group*	*p value*
Pascual [10]	DNS*	DNS*	-
Adas [9]	0	0	NS
Joo [18]	4/30	4/30	NS

* data not shown, NS: nonsignificant

Table 4. The effects of stem cells on the bursting pressure on day 7 after surgery

Study	*Control group*	*Stem cell group*	*p value*
Pascual [10]	115*	125*	NS
Adas [9]	106.5 (80-150)	198.5 (150-260)	<0.001
Joo [18]	121.3	153.92	<0.01

*approximate values

Table 5. The effect of stem cells on adhesions on day 7 after surgery

Study	*Control group*	*Stem cell group*	*p value*
Pascual [10]	8.7	6.2	0.06
Adas [9]	DNS*	DNS*	-
Joo [18]	2.85	2.5	NS

*data not shown, NS: nonsignificant

ing pressure. All the studies monitored the appearance of anastomotic dehiscence, stenosis or dilatation. Pascual et al. [10] and Yoo et al. [11] noticed the severity of the adherence syndrome. The histopathological characteristics of the healing (inflammation, necrosis, the amount of collagen, and angiogenesis) were evaluated [9,11].

Adas et al. [9] proposed administering hydroxyproline in the anastomosis as a method for assessing the strength of the digestive sutures.

Results of individual studies

Because the study designs, interventions and reported outcome measures varied markedly, we focused on describing the studies, their results, and their limitations, rather than meta-analysing them.

As can be seen in Table 3, we found no statis-

Table 6. The effect of stem cells on the histopathological characteristics of gastrointestinal anastomosis healing on day 7 after surgery

Effect	*Adas [9]*	*Joo [18]*
Necrosis	p<0.05	DNS*
Inflammation	NS	0.000
Angiogenesis	NS	0.032
Collagen deposits	p<0.05	0.041
Epithelialisation	NS	DNS*

*data not shown, NS: nonsignificant

tically significant differences between the 2 study groups (with and without stem cells) in terms of the frequency of anastomotic fistula on day 7 after surgery.

The values obtained from calculating the bursting pressure were comparable between the three studies selected (Table 4), and stem cell administration significantly increased anastomosis resistance.

Although Pascual et al. [8] claimed that stem cell administration significantly decreased the formation of adhesions, this could not be demonstrated in other studies (Table 5). Moreover, these differences did not exist in the other groups (day 4, day 14 and day 21).

The results of the statistical analysis of the histopathological characteristics of digestive suture healing are contradictory (Table 6). While Adas et al. [9] found no statistically significant differences between the rate of angiogenesis and inflammation, the study published by Yoo et al. [7] reported a significant increase. In addition, the hydroxyproline content of the anastomoses (proposed by Adas as a marker of the healing) was significantly increased in the stem cell group (0.55 vs 0.89; p <0.001) both on day 7 and on day 4 after surgery.

Discussion

Digestive anastomosis healing is influenced by many general and local factors. Knowledge of these potential risk factors has led to the development of several methods for reducing anastomotic complications, either by controlling the local factors (the use of new techniques for anastomosis or the administration of substances to improve local vascularisation), or by preoperative water-electrolyte and metabolic balancing in order to correct the general factors [12–15].

Mesenchymal stem cells have shown the ability to differentiate into several cell types including osteoblasts, chondroblasts, fibroblasts, adipocytes, skeletal muscle fibres, cardiomyocytes and endothelial cells. This feature may play a role in healing in the intestine and in the amplification of angiogenesis.

In literature there are only three papers studying the way in which stem cells are involved in the healing of gastrointestinal anastomoses. Their results cannot be compared due to increased heterogeneity related to the administration, the number of subjects per group and the follow up period.

Stem cells can be harvested from the bone marrow or from the adipose tissue. There is no evidence that there are differences in their effectiveness depending on the sampling site. The advantages of using multipotent stem cells from the adipose tissue relate to the method of harvest: it is reliable, and there is a low risk of complications. Furthermore, the harvest and extraction of adipose-tissue-derived stem cells is easier and safer than that of bone-marrow-derived stem cells.

The analysis performed revealed that anastomosis resistance was higher in the groups that were administered stem cells, and the only statistically significant findings were in the subjects to which the solutions were injected perianastomotically. Bursting pressure together with dosage of the amount of anastomotic hydroxyproline showed accelerated healing of the anastomosis in the stem cell groups.

It has been shown that the administration of stem cells decreases the incidence of the adherence syndrome without altering the rate of anastomotic dehiscence appearance. Pascual et al. showed that under adhesion-free conditions the resistance of anastomoses is higher in the subjects in which anastomoses are performed with biosutures [8].

Fistula rate was not significantly different between the 2 study groups, which can be explained by the group size and physiopathological features of the experimental model. It is well known that the rat is a reliable model that is resistant to the conditions of sepsis and heals quickly [16].

As far as the histopathological features of the healing are concerned, there are studies proving improved healing after stem cell administration in the ischemic tissue [17]. Our study found a clear increase of the anastomotic resistance due to increased collagen content and acceleration of the angiogenesis processes.

The results were contradictory, which may be due to the fact that the anastomoses were performed under different conditions: normal condi-

tions vs ischemic conditions, with clear benefits in the latter case. Recent studies have demonstrated reperfusion in ischemic tissues after stem cell administration. These properties are the grounds for the use of stem cells in diseases such as ischemic colitis [18,19], acute limb ischemia or acute myocardial ischemia [17,20,21].

Our study has several limitations. The quality of the assessed studies varied. The different size of the study groups and the lack of a confidence interval give the published studies a low level of evidence. Under these conditions, the results should be interpreted with caution. Publication bias might account for some of the properties we observed. Studies on small groups may lead to an overstatement of the effects.

Moreover, the different experimental models (normal vascularisation conditions vs ischemia) and types of stem cell administration - either perianastomotic, or as biosuture - prevented us from performing an appropriate statistical analysis.

Postoperatively, the parameters monitored were different in the three studies, which also prevented the conduct of a thorough meta-analysis.

The inclusion of studies published in English only may be yet another limitation.

Extending the search to applications of the use of stem cells as a method of reducing the incidence of anastomotic complications at other levels of the digestive tract has provided no additional results. For this reason we do not consider that limiting our search to colonic anastomoses influenced our findings.

Overall, the evidence is not sufficiently robust to determine the effectiveness of stem cells in digestive healing. Between 2000 and September 2014 three different experimental studies tried to determine the role of mesenchymal stem cells in reducing the rate of anastomotic leak. All the studies have confirmed a beneficial outcome in terms of anastomotic resistance, although the importance of the effect differed, depending on the study. The estimated effect on the anastomotic leakage was less evident and generated important controversy.

A logical next step for further studies would be to conduct additional, standardized studies to determine the indications for use, the appropriate method of administration, the sampling site and to identify substances whose combination might potentiate their angiogenic effect.

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