Comparative Histological Study on The Effect of Bone Marrow Derived Mesenchymal Stem Cells Versus Wheat Germ Oil on Acute Pancreatitis in A Rat Model

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ABSTRACT

Background: Acute pancreatitis (AP) is a common inflammatory disorder of digestive system. Mesenchymal stem cells (MSCs) and Wheat germ oil (WGO) could improve AP through their anti-inflammatory and antioxidant effects.

Objective: Evaluate and compare the possible therapeutic effects of Bone marrow derived Mesenchymal Stem Cells (BMSCs) versus WGO on AP.

Materials and Methods: 47 adult male albino rats were divided into 4 groups. Control group I (no.=12). AP was induced in the remaining 35 rats by a single intra peritoneal (I.P) injection of L-arginine (250 mg/100g). 5 rats died within the 1st hour after AP induction, the rest were divided randomly into group II (AP group; no.=10) that received no treatment, group III (BMSCs group; no.=10) and group IV (WGO group; no.=10). One hour after AP induction, group III was injected I.P. by 1ml of PKH26 labeled BMSCs (1x106 cells/ml) and group IV received WGO in a dose of 3 ml/kg body weight by oral gavage every 24 h for 3 successive days. Blood samples were collected 24 hours and on the 4th day after AP induction for biochemical assessment of serum amylase, lipase, interleukin-1β and interleukin-10. Then, animals were sacrificed and specimens from the pancreas were prepared for Hematoxylin and eosin (H&E) stain and immune-histochemical staining using inducible Nitric Oxide Synthase (iNOS) and insulin antibodies. Morphometric measurements using image analyzer were done.

Results: Group II showed extensive pancreatic damage associated with increase in serum amylase, lipase and interleukin-1B levels and reduction in interleukin-10 level. A significant increase in the area % of iNOS immunostaining and non-significant change in insulin immunostaining were detected. On the other hand, BMSCs group and WGO group showed improvement in the biochemical, histological and immunohistochemical results with better results in BMSCs group.

Conclusion: BMSCs possess better therapeutic efficacy in treating AP compared with WGO.

INTRODUCTION

Acute pancreatitis (AP) is a common inflammatory disease. Most of cases show self-limited systemic inflammatory of mild acute pancreatitis (MAP). However, 10-15% of cases show severe acute pancreatitis (SAP) with infection, pancreatic necrosis, multiple organ failure and mortality rate 30-47%.[1 & 2].

There is mainly supportive but not specific treatment for acute pancreatitis despite its increasing incidence[3]. For example, drainage, debridement and antibiotics for treatment of infected pancreatic necrosis[4].

The early activation of intracellular pancreatic enzymes has been the basic mechanism of AP. However, other mechanisms such as oxidative stress, impaired autophagy have been involved[5].

Mesenchymal stem cells (MSCs) could manage AP. They differentiate in vitro into different tissues under suitable conditions[6]. They home to inflammatory tissues, secret anti-inflammatory cytokines that modulate the immune response in addition; they have antioxidant effect[7 & 8].

Wheat germ oil (WGO) is a good antioxidant due to its high content of tocopherols (particularly vitamin E) and carotenoid. Moreover, it has anti-inflammatory effect because of its ceramide content and it contains antimicrobial compounds[9].

This study aimed to evaluate and compare the possible therapeutic effects of Bone marrow derived Mesenchymal Stem Cells versus wheat germ oil on acute pancreatitis induced by L-Arginine in a rat model using biochemical, histological, immunohistochemical and morphometrical methods.
MATERIALS AND METHODS

Animals

Forty-seven adult male albino rats (180-200 gm) were housed and treated in Animal House, Faculty of Medicine, Cairo University according to the guidelines approved by the Animal Use Committee of Cairo University. Rats received ordinary rat chow, bred. They were housed in properly ventilated wire cages at temperature (24 ± 1°C), with normal light/dark cycle. All animals had free access to food and water and followed the same environmental conditions.

Chemicals

- L-arginine: L-arginine (L-arg.) powder (L-Arginine – reagent grade, ≥ 98%, Sigma Chemical CO., P.O. Box 14508 St. Louis, MO 63178 USA, A-5006 USA).
- Wheat germ oil: Wheat germ oil (WGO) was purchased from El-Captain Company (CAP PHARMA, Al Obor City, Egypt), as a bottle of 30 ml WGO in a liquid form.
- Chemicals for immunostaining:
  - Primary antibodies:
    - Insulin Monoclonal Antibody (ICBTACLS), eBioscience™: It is mouse monoclonal antibody (Thermo Fisher Scientific 10255 Science Center Drive San Diego, CA 92121, catalogue number 14-9769-82)
    - Anti iNOS antibody: It is a rabbit polyclonal antibody (Thermo Fisher Scientific 3747 N. Meridian Road Rockford, IL 61105 USA, catalogue number PA1-21054).
  - Citrate buffer (cat no AP 9003), Ultravision detection system (Anti-Polyvalent, HRP/DAB Kit detection system, cat no TP - 015-HD) and Mayer’s hematoxylin (cat no TA-060-MH). All were purchased from Thermo Fisher Scientific, USA.

Preparation of Bone Marrow Derived Mesenchymal Stem Cells from Rats

Isolation, culture and labeling of MSCs were done at Biochemistry and Molecular Biology Unit at Biochemistry department, Faculty of Medicine, Cairo University, Egypt\(^{[10]}\).

MSCs in culture were characterized by their plastic adhesiveness and fusiform shape\(^{[11]}\).

Immunophenotyping of separated cells

- Using flow cytometry (Accuri, USA), the MSCs were positive for CD29 (Sigma, USA, SAB 4501582) and negative for CD45 (Sigma, USA, OX-1 84112004)\(^{[12]}\).

Cell Viability Analysis

Using trypan blue dye, the viable cells appeared shinny unstained under the microscope, while dead cells appeared blue\(^{[13]}\).

Labeling of MSCs with PKH26 dye

Cultured cells were labeled with fluorescent cell tracker PKH26 (Sigma, USA, MINI26) according to manufacturer’s instructions to track their migration and homing\(^{[14]}\).

Experimental design

Group I (Control Group (C); no.=12)

This group included three equal subgroups:
- Subgroup IA: received 2.5 ml saline (El Fath for Drug and Cosmetics Industry, FIPCO, New Borg Al Arab City, Egypt) by intra peritoneal (I.P.) injection once.
- Subgroup IB: received 2.5 ml saline by I.P. injection once then after 1h received 1ml Phosphate Buffer Saline (PBS) (Sigma Chemical CO.P.3813 USA). by I.P. injection.
- Subgroup IC: received 2.5 ml saline by I.P. injection once then after 1h received 3 ml/kg body weight (0.6ml / rat) distilled water by oral gavage every 24 hours for 3 successive days.

Group II (Acute Pancreatitis Group (AP); no.=10)

This group was injected I.P. by 2.5 ml L-arg. 20% concentration\(^{[15]}\). They were subdivided into two equal subgroups:
- Subgroup IIA (5 rats): 1h after AP induction received 1ml PBS by I.P. injection.
- Subgroup IIB (5 rats): 1h after AP induction received 3 ml/kg body weight (0.6 ml/ rat) distilled water by oral gavage every 24 hours for 3 successive days.

Group III (Bone marrow derived Mesenchymal Stem Cells Group (BMSCs); no.=10)

This group received the same dose of L-arg. as AP group then after 1h they were injected I.P. by 1ml /rat of PKH26 labeled BMSCs (1x106 cells/ ml) suspension in PBS\(^{[16]}\).

Group IV (Wheat Germ Oil Group (WGO); no.=10)

This group received the same dose of L-arg. as AP group then after 1h they received WGO in a daily dose of 3 ml/kg body weight (0.6ml/rat) by oral gavage every 24 hours for 3 successive days\(^{[17]}\).
Rats were sacrificed on the 4th day after AP induction.

**Experimental procedure**

**1-Induction of Acute Pancreatitis**

L-arginine 20% solution was prepared by dissolving 2g L-arginine hydrochloride in 8ml 0.9% saline. The pH was adjusted to 7 and volume was completed to 10 ml with saline. It was prepared in Biochemistry Department, Faculty of Medicine, Cairo University. It was injected I.P. in a dose of 2.5 ml L-arg. 20% concentration (A dose of 250 mg/100 g body weight, equivalent to 1.25 ml/100 g body weight). Fresh solution was prepared on the day of injection\(^1\). Acute pancreatitis was induced in thirty-five rats. Five rats died within the 1st hour after AP induction and the rest were divided randomly into 3 groups.

**2-Treatment of Acute Pancreatitis**

- Treatment with BMSCs: allogeneic PKH26 florescent labeled BMSCs suspension in PBS, were injected I.P. in a dose of 1ml/rat (1x10⁶ cells/ml) 1h after AP induction\(^2\).
- Treatment with WGO: 1h after AP induction WGO in a dose of 3 ml/kg body weight (0.6ml/rat) was given by oral gavage then every 24h for 3 successive days\(^3\).

**3-Laboratory Investigation**

Blood samples from retro orbital vein were collected 24h and on the 4th day after AP induction to assess levels of serum amylase, lipase, interleukin-1B and interleukin-10. They were measured in the Biochemistry Department, Faculty of Medicine, Cairo University.

**4-Light microscopic studies**

The animals were sacrificed on the 4th day after AP induction using chloroform inhalation. The pancreas was dissected out, fixed in 10 % formol saline for 24 hours at room temperature, dehydrated in ascending grades of alcohol, cleared in xylene then embedded into paraffin wax (Histology Department, Faculty of Medicine, Cairo University).

Sections of 5 μm thickness were stained with the following stains:

- a. Hematoxylin and Eosin\(^4\).
- b. Immunohistochemical staining\(^5\) using:
  1. Anti inducible Nitric Oxide Synthase (iNOS) antibodies.
  2. Anti insulin antibodies.

Immunostaining for iNOS only required pretreatment for antigen retrieval, by boiling for 10 minutes in 10Mm, pH 6 citrate buffer and cooling sections for 20 minutes in room temperature. Sections were incubated with the primary antibodies for 1 hour. Ultravision detection system was used and counterstaining was done using Mayer's hematoxylin. iNOS and insulin Positive reactions appear as brown cytoplasmic deposits.

**c- Fluorescent microscopic study**

Unstained sections of group III were examined by fluorescent microscope (Olympus BX-51 F4, Olympus Optical Co. Ltd., Japan. No.7 MO3285) to detect homing of PKH26 labeled BMSCs. PKH26-labeled Cells appear as red to yellow fluorescense according to the intensity of labeling.

**d- Morphometric study: It included**

- Mean area percent of iNOS immuno-expression in iNOS immunostained sections at a magnification of ×100.
- Mean area percent of insulin immuno-expression in insulin immunostained sections at a magnification of ×100.

All measurements were done in 10 non overlapping fields from different sections of each group. Image analysis was done using "Leica Qwin 500 C" software image analysis computer system (Cambridge, England) in image analyzing unit, Histology and cell Biology Department, Faculty of Medicine, Cairo University.

**e- Statistical Analysis**

The morphometric and biochemical measurements were expressed as mean ± standard deviation (SD) and were analysed using the software “SPSS” version 20. This was done using ANOVA followed by “Tukey” post hoc test. Results were considered significant when P value was < 0.05\(^6\).

**RESULTS**

**General Observation**

Five rats died within the first hour after the intraperitoneal (I.P.) injection of L-arginine and the other rats became less active regaining their activity on the second day.

The biochemical, histological and immunohistochemical results of all subgroups of control group were similar. The AP rats in subgroup IIA and IIB also showed the same biochemical, histological and immunohistochemical results. Therefore, they were referred to by group I (control group) and group II (AP group), respectively.

**A) Lab Results**

**I- Plasma Amylase, Lipase and IL-1β Levels (Histogram 1 & 2)**

After 24 h and on 4th day of AP induction, the mean values of plasma amylase, lipase and IL-1β levels showed significant increase in groups GII, GIII and GIV compared to control. There was significant decrease in these mean values in GIII and GIV when compared with GII while there was significant increase in GIV when compared with GIII.
IL-10 Level (Histogram 3)

After 24 h and on 4th day of AP induction, the mean value of plasma IL-10 level showed significant decrease in groups GII, GIII and GIV compared to control. There was significant increase in the mean value of plasma IL-10 level in GII and GIV when compared with GII while there was significant decrease in GIV when compared with GIII.

B) Histological Results

Fluorescent Microscope Results

BMSCs group (GIII) showed the presence of PKH26 labeled stem cells in connective tissue (C.T) septa and pancreatic acini (Figure 1).

Hematoxylin and Eosin Stained Pancreatic Sections

Control group showed normal structure of the pancreas (Figure 2). The AP group showed disorganized acinar architecture. Some acinar cells appeared apoptotic with dark pyknotic nuclei and deep acidophilic cytoplasm. Other cells show cytoplasmic vacuolations. In addition, there was infiltration with Inflammatory cells (Figure 3). However, the acinar architecture was preserved in the acini surrounding the apparently normal islet (Figure 4). BMSCs Group showed an almost apparently normal pancreatic architecture (Figure 5) while WGO Group showed apparently normal acinar pattern with some distorted acini. Less apical acidophilia was noted in some acinar cells and Few cytoplasmic vacuoles were seen (Figure 6).

Anti-iNOS stained pancreatic sections

Control group revealed negative immunoreactivity (Figure 7). AP Group showed marked cytoplasmic dark brown granules of most acini. Few islet cells showed immunoreaction (Figure 8). BMSCs Group showed weak cytoplasmic reactivity in acinar cells and few reactive cells mainly in between acini (Figure 9). WGO Group revealed moderate cytoplasmic reactivity in most acini. Few cells, mainly in between acini and few islet cells were immune reactive (Figure 10).

Anti-insulin stained pancreatic sections

Pancreatic sections of GI, GII, GIII and GIV showed dense brown cytoplasmic immunoreactivity in most cells of the pancreatic islets of Langerhans (Figures 11-14) respectively.

C) Morphometric results

I-Mean area percent of iNOS immunoreactivity (Table 1)

The mean area percent of iNOS immunoreactivity in GIII and GIV showed significant decrease when compared to GII, while there was significant increase in GIV when compared with GIII.

II-Mean area percent of insulin immunoreactivity (Table 2)

There is no significant difference between the mean values of area % of insulin immunoreactivity in the control and the experimental groups.
Fig. 4: A photomicrograph of a section in the pancreas of an albino rat from AP group (GII) showing destruction of the normal pancreatic architecture (Asterix) with occasional preservation of some acini (A) surrounding an apparently normal islet of langerhans (I). (H&E x 200)

Fig. 5: A photomicrograph of a section in the pancreas of an albino rat from BMSCs treated group (GIII) shows apparently normal architecture of pancreatic acini. (H&E x 200)

Fig. 6: A photomicrograph of a section in the pancreas of an albino rat from WGO treated group (GIV) shows apparently normal architecture. Some acini are distorted (arrows), others show less apical acidophilia (curved arrows) and few cells appear vacuolated (v). (H&E x 200)

Fig. 7: A photomicrograph of a pancreatic section of control group (GI) showing -ve immunoreactivity for iNOS. (anti iNOS x 200).

Fig. 8: A photomicrograph of a pancreatic section of AP group (GII) showing strong immunoreactivity for iNOS in most pancreatic acini (A). Few cells in the islet (I) show immune reactivity (arrows). (anti iNOS x 200).

Fig. 9: A photomicrograph of a pancreatic section of BMSCs group (GIII) showing weak immunoreactivity in acinar cells (A) for iNOS. Few immune reactive cells are seen in between acini (arrows). (anti iNOS x 200).
Fig. 10: A photomicrograph of a pancreatic section of WGO group (GIV) showing moderate immunoreactivity for iNOS in most pancreatic acini (A). Few islet cells are immune reactive (arrow heads). Few immune reactive cells are seen in between the acini (arrows). (anti iNOS x 200).

Fig. 11: A photomicrograph of a pancreatic section of control group (GII) showing dense cytoplasmic immunoreactivity in one islet of Langerhans. The immunoreactivity is observed in most islet cells. (Anti-insulin Immunostaining x 200).

Fig. 12: A photomicrograph of a pancreatic section of AP group (GII) showing dense cytoplasmic immunoreactivity in one islet of Langerhans. The immunoreactivity is observed in most islet cells. (Anti-insulin Immunostaining x 200).

Fig. 13: A photomicrograph of a pancreatic section of AP group (GIII) showing dense cytoplasmic immunoreactivity in one islet of Langerhans. The immunoreactivity is observed in most islet cells. (Anti-insulin Immunostaining x 200).

Fig. 14: A photomicrograph of a pancreatic section of AP group (GIV) showing dense cytoplasmic immunoreactivity in one islet of Langerhans. The immunoreactivity is observed in most islet cells. (Anti-insulin Immunostaining x 200).

Table 1: The mean values (±SD) of area % of iNOS immunoreactivity in experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ±SD</th>
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<tbody>
<tr>
<td>GII</td>
<td>30.1 ± 2.3</td>
</tr>
<tr>
<td>GIII</td>
<td>7.04 ±0.8*</td>
</tr>
<tr>
<td>GIV</td>
<td>10.8 ± 0.9**</td>
</tr>
</tbody>
</table>

* there is significant decrease when compared with GII.
** there is significant increase when compared with GIII.

Table 2: The mean values (±SD) of area % of insulin immunoreactivity in control and experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>50.3 (± 4.9)</td>
</tr>
<tr>
<td>GII</td>
<td>47.5 (± 3.2)</td>
</tr>
<tr>
<td>GIII</td>
<td>51.4 (±3.8)</td>
</tr>
<tr>
<td>GIV</td>
<td>51.2 (± 2.8)</td>
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</table>
The current study induced experimental AP in rats by using a single I.P injection of L-arginine in a dose of 250 mg/100g body weight. This is in accordance with El-Rahman et al. (2011)\textsuperscript{[21]} and Hasan et al. (2015)\textsuperscript{[22]}. L-arginine produces biochemical and histological changes like that of human AP\textsuperscript{[23]}. It distorts the membranes of zymogen granules and release their enzymes to the interstitium causing inflammation\textsuperscript{[24]}. Time of scarification was chosen according to Moreira et al., 2011\textsuperscript{[24]} who stated that peak histological changes were observed around 72 h after induction of AP.

In this study, the mortality rate in the 1st hour after AP induction in GII (AP), GIII (BMSCs) and GIV (WGO) was 14.3%. The Overall mortality in AP is about 3%–6%. Whereas, it reaches 30% in severe acute pancreatitis (SAP) due to absence of specific treatment\textsuperscript{[25]}

The pancreatic enzymes derived from pancreatic acini (amylase, lipase, and the proenzyme trypsinogen) are considered the main diagnostic parameters of AP\textsuperscript{[26]}. In this study, there was significant elevation in serum amylase and lipase 24 h and on 4th day following AP induction in AP group as compared with control group. These findings are in accordance with Abdin et al., 2010\textsuperscript{[27]}, El-Rahman et al., 2011\textsuperscript{[28]} and Yin et al., 2015\textsuperscript{[29]}. This may be due to membrane disruption of zymogen granules secondary to increased free radicals leading to release of amylase and lipase into interstitium\textsuperscript{[30]}. It also, could be secondary to elevation of inflammatory cytokines and lipid peroxidation\textsuperscript{[30]}. Shah et al., 2010\textsuperscript{[30]} mentioned that, the serum amylase in AP elevates within 6 hours of AP onset and remains elevated for 3–5 days. Lippi et al., 2012\textsuperscript{[31]} stated that serum lipase elevates within 3–6 hours of AP onset and remains elevated for around 7–14 days.

In the present work, there was significant elevation in serum IL-1β 24h and on 4th day after AP induction in the AP group as compared with control group. This agrees with Jung et al., 2015\textsuperscript{[32]} who observed the increase in pro-inflammatory cytokines in SAP, such as TNF-a, IL-1β, IL-6, and other inflammatory mediators, including iNOS. Xu et al 2014\textsuperscript{[33]} stated that IL-1β activates trypsin and impairs autophagy by changing the intracellular calcium leading to decrease in the viability of acinar cells. Autophagy includes lysosome-mediated processing, elimination of damaged proteins, organelles or microorganisms\textsuperscript{[33]}. Grasso et al., 2011\textsuperscript{[34]} considered autophagy as a protective process that damages harmful activated zymogens during early pancreatitis. On the other hand, Hashimoto et al., 2008\textsuperscript{[35]} suggested that autophagy damages acinar cells by delivering trypsinogen to the lysosomes, which activate it to trypsin. Mareninova et al., 2009\textsuperscript{[36]} showed that impaired autophagy is associated with imbalance between degradation and activation of trypsinogen, leading to intra-acinar accumulation of active trypsin. Moreover, autophagy in acute pancreatitis includes degradation of damaged mitochondria in a process called Mitophagy\textsuperscript{[37]}

In this study, serum IL-10 was reduced 24h and on 4th day after AP induction in the AP group as compared with control group. This was in accordance with Liu et al., 2014\textsuperscript{[38]} who found decrease in serum IL-10 in cerulein-induced SAP mice. IL-10 is anti-inflammatory cytokine that correlates with the severity of pancreatitis. It decreases the inflammatory cytokines, serum amylase, serum lipase, edema, necrosis and hemorrhage in AP. Moreover, IL-10 can improve the severity of caerulein-induced AP when given as prophylaxis and treatment\textsuperscript{[4 and 39]}. 

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**THE EFFECT OF BMSCS VERSUS WGO ON ACUTE PANCREATITIS IN RAT MODEL**

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**DISCUSSION**

The current study induced experimental AP in rats by using a single I.P injection of L-arginine in a dose of 250 mg/100g body weight. This is in accordance with El-Rahman et al. (2011)\textsuperscript{[21]} and Hasan et al. (2015)\textsuperscript{[22]}. L-arginine produces biochemical and histological changes like that of human AP\textsuperscript{[23]}. It distorts the membranes of zymogen granules and release their enzymes to the interstitium causing inflammation\textsuperscript{[24]}. Time of scarification was chosen according to Moreira et al., 2011\textsuperscript{[24]} who stated that peak histological changes were observed around 72 h after induction of AP.

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**Histogram 1:** The mean values of plasma Amylase & Lipase levels of control and experimental groups 24h and 4th day after AP induction

The mean difference is significant at the 0.05 level.
- * Significant increase when compared with GI.
- * Significant decrease when compared with GII.
- ■ Significant increase when compared with GIII.

**Histogram 2:** The mean values of plasma IL-1B level of control and experimental groups 24h and 4th day after AP induction

The mean difference is significant at the 0.05 level.
- * Significant increase when compared with GI.
- * Significant decrease when compared with GII.
- ■ Significant increase when compared with GIII.

**Histogram 3:** The mean values of plasma IL-10 level of control and experimental groups 24h and 4th day after AP induction

The mean difference is significant at the 0.05 level.
- * Significant increase when compared with GI.
- * Significant decrease when compared with GII.
- ■ Significant increase when compared with GIII.
MSCs could repair tissues in acute diseases and have shown improving effects on different acute injuries. In this study, using BMSCs in GIII significantly decreased both plasma amylase and lipase levels after 24 h and on 4th day. This was coincident with Yin et al., 2015 and Qu et al., 2017. Transplanted BMSCs could develop into pancreatic cells in vivo. Moreover, they could reduce inflammation by decreasing cytokines in AP and IL-6 but increased that of IL-10 in SAP. Treatment with BMSCs in GIII was associated with significant elevation of serum IL-10 24h and on 4th day after AP induction as compared with AP group. These results are in line with the study of Meng et al., 2013 who demonstrated that human BMSCs decreased the level of IL-1β in SAP. In addition, Tu et al., 2012 showed the same finding by using umbilical cord-derived MSCs. Jung et al., 2015 suggested that human BMSCs improve SAP secondary to its anti-inflammatory effect. Treatment with BMSCs in GIII was associated with significant elevation of serum IL-10 24 h and on 4th day after AP induction as compared with AP group. This was in accordance with Tu et al., 2012 who found that treatment with BMSCs suppressed the high level of TNF and IL-6 but increased that of IL-10 in SAP.

Until now, the role of WGO in L-Arginine-induced AP has not been reported except for the study done by Abdel-Gawad, 2015. In the current study using WGO in the treatment of AP decreased levels of both serum amylase and lipase 24 h and on 4th day after AP induction but these results disagreed with Abdel-Gawad, 2015 who found that increased serum lipase activity after 24h was not affected by treatment with WGO. In addition, in WGO treated group (GIV) there was significant reduction in IL-1β as compared with AP group, which is in line with Abdel-Gawad, 2015 who found that WGO treatment decreased the elevated level of pancreatic IL-1β in the treated group. Treating with WGO caused elevation in serum IL-10 24 h and on 4th day after AP induction. This could be due to the high content of vitamin E in WGO that was in line with Xiong et al., 2014 who indicated that vitamin E significantly increased the level of IL 10 in arthritis in rats. In addition, this result agreed with EL-Feki et al., 2016 who observed that Pre-treatment with vitamin E increased IL-10 level in hepatotoxicity in rats. Wheat germ oil can reduce oxidative stress through vitamin E that protects DNA and cell membrane from oxidative damage. Furthermore, wheat germ oil has anti-inflammatory effects through Omega-3 unsaturated fatty acids.

In this current study, detection of I.P. injected BMSCs labeled with PKH26 fluorescent dye 24 h after AP induction in pancreatic tissue indicates homing of these cells into the pancreas. On contrary Dong et al., 2018 found nearly no BMSCs in pancreas after its I.P. injection while they found more BMSCs after its injection either through tail vein or through both tail vein and peritoneum. However, they suggested that the protective role of I.P injected BMSCs was done without homing to pancreas through a paracrine effect. MSCs could secret some anti-inflammatory cytokines in a paracrine manner. For example, they secret interleukin 10, insulin-like growth factor (IGF-1) and vascular endothelial growth factors.

Histological examination of HandE stained pancreatic sections of AP group showed disturbed acinar architecture with inflammatory cell infiltration. Apoptotic cells with dark pyknotic nuclei and deep acidophilic cytoplasm were also seen. These results were in accordance with Qu et al., 2017 who found structural distortion, inflammatory cell infiltration, hemorrhage and necrosis in AP. In addition, Jung et al., 2015 found marked inflammation, edema and necrosis in AP group.

The apoptosis of pancreatic acinar cells is secondary to inflammation induced by L-arginine. This might be due to metabolic changes in the endoplasmic reticulum and inhibited synthesis of nucleic acids. In addition, it could be due to disturbance of protein synthesis due to decreased polyamine synthesis. Moreover, L-arginine can produce excess nitric oxide (NO) leading to vascular dilatation, blood stasis and inflammatory infiltration. Furthermore, oxygen free radicals and inflammatory cytokines are involved in the activation of L-arginine induced AP.

In this study cytoplasmic vacuolations were detected which agreed with the results of El-Rahman et al., 2011 who found multiple vacuoles within pancreatic acinar cells. Mareninova et al., 2009 suggested that these vacuoles were due to disturbed autophagy occurred in AP. Autophagy was caused by decreased lysosomal degradation secondary to imbalance between cathepsin L and cathepsin B. Cathepsin L degrades trypsinogen and trypsin while cathepsin B converts trypsinogen into trypsin. Finally, this imbalance leads to intra-acinar accumulation of active trypsin. Ropolo, 2007 suggested that during AP, a transmembrane protein called Vacuole Membrane Protein 1(VMP1) was highly expressed in acinar cells leading to numerous cytoplasmic vacuoles.

In this study, the acinar architecture was intact in the acini surrounding the islet, which was in line with Abdel-Gawad, 2015. Moreover, it agreed with Hegyi et al., 2004 who observed that acinar cells surrounding the islets of Langerhans remained intact. To confirm their finding, they induced pancreatitis in diabetic rats and found that, acini surrounding islets did not remain intact. They also found decreased pancreatic regenerative response to exogenous cholecystokinin (CCK-8) and explained that by the low insulin level. Moreover, they found no histological difference between the acini surrounding the islets and that far from it. Their findings confirmed the role of insulin in the regulation of the exocrine pancreatic structure.

In this study, treating with stem cells caused improvement in the HandE stained pancreatic sections in BMSCs treated group showing an almost normal pancreatic architecture. Most of the acini appeared normal with basal basophilia and apical acidophilia as well as apparently normal zymogen granules. This was in accordance with Zhao et al., 2016 who found that after BMSCs...
transplantation, the pancreatic necrosis and inflammation were significantly improved. Jung et al., 2011[40] and Meng et al., 2013[42] have reported that human BMSCs and human umbilical cord-derived MSCs, decrease serum and pancreatic levels of pro-inflammatory cytokines and increase anti-inflammatory cytokines. Yang et al., 2013[34] stated that umbilical cord MSCs transplanted immediately after induction of AP showed a better anti-inflammatory effect than those transplanted several hours after induction. Jung et al., 2015[32] and Kawakubo et al., 2018[39] reported that the anti-apoptotic and anti-inflammatory effect of BMSCs could occur through several molecules secreted by MSCs. Yin et al., 2016[30] showed that micro vesicles from BMSCs attenuated injury of AP. He et al., 2016[77] and Zhao et al., 2016[33] have described that BMSCs could migrate to several organs including the pancreas.

Examining sections of the WGO treated group showed improvement in the histological picture in the form of apparently normal acinar pattern with some distorted acini. Some acini showed less apical acidophilia in the acinar cells. These results agreed with Abdel-Gawad, 2015[17] who found that WGO improved L-arginine-induced AP through its anti-inflammatory effect which might be through inhibiting the release of inflammatory cytokine IL-1β.

In current study immunohistochemical examination for iNOS showed its increased expression in AP group as compared with control group and that agrees with Choi et al., 2016[30] who found that the iNOS was markedly expressed in mice at 72 h after induction of pancreatitis by L-arginine. Moreover, few immune reactive cells were seen in between the acini, this come in line with Buchwalow et al., 2013[33] who found increased iNOS expression in pancreatitis with strong immunostained infiltrating inflammatory cells adjacent to the affected areas. Nitric oxide is one of reactive nitrogen species associated with oxidative stress that plays a principle role in AP. Nitric Oxide could have a beneficial effect, however, its over production could be very harmful.[80]

Nitric oxide plays an important role in signaling, proliferation, survival, and death of both normal and transformed cells[81]. Nitric oxide synthase (NOS) enzyme produces nitric oxide in cells by converting l-arginine into l-citrulline and NO[82]. There are three NOS isoforms encoded by three different genes. The constitutive (cNOS) and Ca+2-dependent isoforms include neuronal (nNOS) encoded by NOS1gene. The endothelial (eNOS) encoded by NOS3 gene. The Ca+2-independent inducible nitric oxide synthase (iNOS) encoded by NOS2 gene. Inducible nitric oxide synthase is induced by inflammatory cytokines, endotoxin and hypoxia[83]. Constitutive eNOS generates NO in little concentration in physiological conditions, while iNOS can produce NO at much higher concentration with sustained activity in pathological conditions[84].

In this study, treatment with BMSCs markedly reduced the iNOS expression as confirmed by significant decrease in iNOS area percent in BMSCs treated group when compared with AP group. This was in line with yang et al., 2015[64] who indicated the beneficial effect of BMSCs derived extracellular vesicles (EVs) through decreasing the proinflammatory mediators such as TNF-α and iNOS. They stated that, the expression of iNOS and TNF-α significantly decreased in colitis after treatment with BMSC-EVs. Jung et al., 2015[32] suggested that human BMSCs improved SAP by its anti-inflammatory effect. Inan et al., 2017[83] hypothesized that MSC homed to the site of injury in intestinal ischemia/reperfusion model and decreased oxidative stress. They explained this anti-oxidative effect by decreasing oxygen radicals and pro-inflammatory cytokines meanwhile increasing anti-inflammatory cytokines.

In this study, treatment with WGO reduced the iNOS expression as confirmed by significant decrease in iNOS area % in WGO group compared with AP group. This was to a lesser extent than BMSCs as confirmed by significant increase in iNOS area % in WGO group when compared with BMSCs group. This result come in line with Akool., 2015[66] who found that administration of WGO significantly reduced iNOS expression in cyclosporin A -induced hepatotoxicity in rats. He mentioned that WGO could restore the balance between oxygen radical production and endogenous antioxidant defense system that was disturbed by cyclosporin A.

In this study, immunohistochemical examination for insulin revealed no affection to the islets of Langerhans in the AP, BMSCs and WGO groups as they showed positive reaction like that of the control group. This was confirmed by nonsignificant difference in insulin area percent between the control and the experimental groups. This result come in line with Kovalska et al., 2012[87] who found that the microscopic structure of the islets of Langerhans was intact among masses of debris in patients with acute necrotizing pancreatitis. In addition, staining with Masson showed a net of fibrin surrounding the islets of Langerhans that protected its structure in patients with acute necrotizing pancreatitis.

CONCLUSION

In this study, the improving effect of BMSCs on AP was superior to the effect of WGO as confirmed by biochemical, histological and immunohistochemical methods.

CONFLICT OF INTEREST

There are no conflicts of interest

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دراسة هستولوجية على تأثير الخلايا الجذعية المستخرجة من نخاع العظام
مقارنة بزيت جنين القمح على إلتهاب البنكرياس الحاد في نموذج الجرذ
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الخلفية والأهداف: إلتهاب البنكرياس الحاد (AP) هو إصابة شائعة في الجهاز الهضمي. يمكن للخلايا الجذعية الوسيطة (MSCs) وزيت جنين القمح (WGO) تحصين إلتهاب البنكرياس الحاد من خلال تأثيرهما المضاد للالتهاب ومضاد للأكسدة.

الهدف: تقييم ومقارنة التأثيرات العلاجية المحتملة للخلايا الجذعية الوسيطة المستمدة من نخاع العظام (BMSCs) مقابل بزيت جنين القمح على إلتهاب البنكرياس الحاد.

طرق البحث والنتائج:
- تم تقسيم 42 من الفئران البيضاء الذكور البالغين إلى 4 مجموعات. المجموعة الضابطة (I.P) المكونة من الفئران التي لم تتلق أي علاج. المجموعة الثانية (مجموعة AP) المكونة من الفئران التي تلقت بزيت جنين القمح.
- المجموعة الثالثة (مجموعة BMSCs) المكونة من الفئران التي تلقت انتزاع وخلايا جذعية وسية من نخاع العظام. المجموعة الرابعة (مجموعة WGO) المكونة من الفئران التي تلقت زيت جنين القمح مع انتزاع وخلايا جذعية وسية من نخاع العظام.

تقرير الحالات: في المجموعة الضابطة، لم تظهر أي تأثيرات علاجية. في المجموعة الثانية، تحسنت مستويات الأنزيمات وانخفاض مستويات البروتينات. في المجموعة الثالثة، تضاعفت نسبة منطقة الصبغة المناعية لـ iNOS. في المجموعة الرابعة، تظهرت نتائج أفضل من حيث الفعالية العلاجية والأمان.

الخلاصة: تمكّنت BMSCs من تقديم نتائج أفضل في علاج AP مقارنة مع WGO وزيت جنين القمح. يظهر أن استخدام الخلايا الجذعية الوسية من نخاع العظام ك liệu يمكن أن يكون فعالًا في علاج إلتهاب البنكرياس الحاد.