Treatment of Experimentally-Induced Peptic Ulcer in Rats by Hematopoietic Stem Cells

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Abstract

Regenerative medicine and emerging biotechnologies has encouraged the use of stem cells for the repair of injured tissues and organs, including the stomach. This study aims to examine the therapeutic effect of hematopoietic stem cells (in the treatment of experimentally induced peptic ulcer in rats. Thirty adult male albino rats weighing 150-200 gram were used. The rats were divided into three equal groups. (1) Control group received 1ml/rat single dose of 0.9% saline via a gastric tube. (2) Peptic ulcer non-treated group received 80% ethanol (1ml/rat) via a gastric tube. (3) Peptic ulcer treated group rats were treated with a single dose of CD34 positive HSCs (2x10^6 cell/rat intravenously) 48 hour following the induction of peptic ulcer. After 4 weeks all the rats were sacrificed and the gastric juice was collected. The stomach was excised for histopathological and immunohistochemical studies. The treatment with HSCs resulted in significant decrease in the volume, free acidity and total acidity of gastric juice when compared to the corresponding values in the non-treated group. Histopathological examination revealed that most of the luminal surface of the gastric mucosa was intact, with close similarity to the microscopic picture of the control group. This study shows that the use of HSCs could be a promising line of treatment for peptic ulcer, especially the complicated or recurrent cases.

Key Words: Peptic ulcer – Stem cell – Gastric mucosa – Gastric juice – Ethanol.

Introduction

Under normal conditions, a physiological balance exists between acid secretion of stomach and natural gastroduodenal mucosal defense. Peptic ulcer, which is an eroded part of the gastric mucosa, may occur when the above-mentioned physiological balance is disturbed [1]. Many factors, such as non steroidal anti-inflammatory drugs (NSAIDs), helicobacter pylori (H. pylori), alcohol abuse, bile salts and acidifying salts are reported to change the gastro-duodenal mucosal defense with subsequent development of peptic ulcer. Also, stress, cigarette smoking and spices are known to be contributing causative factors for peptic ulcer development [2]. The above-mentioned factors make the exact pathophysiological mechanism of peptic ulcer to be a matter of debate and in turn diverse strategies of treatment for such disease are encountered. The treatment policy of all drugs prescribed for peptic ulcer aimed at decrease acid and/or induce the epithelial mucosal cells to regenerate again [3]. However, no known available drug is able to induce epithelial cell replication [4]. Recently, stem cells are introduced in the therapeutic medical field where they are used in some degenerative diseases like Parkinsonism, Alzheimer and diabetes [5]. Peptic ulcer is a prevalent and clinically significant condition with important implications on health care costs worldwide. Negative outcomes include bleeding, perforation and even death are associated with decompensation of coexisting medical conditions [2]. Due to problems associated with recurrence after treatment, there is a need to seek alternative drug sources against GI ulcers [6]. Stem cells in the adult body may be useful for recovering the lost functions of damaged tissues especially gastrointestinal tract mucosa [7]. Recent studies have demonstrated that stem cells have greater plasticity and can differentiate down multiple cell lineages in rodents [8]. Regenerative medicine and emerging biotechnologies stand to revolutionize the practice of medicine. Advancesments in stem cell biology, including embryonic and postnatal somatic stem cells, have made the prospect of tissue regeneration a potential clinical reality [9]. Medical researchers believed that stem cell therapy has the potential to dramatically change the treatment of human disease. A number of adult
stem cell therapies already exist, particularly bone marrow transplants that are used to treat leukemia [10]. In the future, medical researchers anticipate being able to use technologies derived from stem cell research to treat a wider variety of diseases including cancer, Parkinson’s disease, spinal cord injuries, amyotrophic lateral sclerosis, multiple sclerosis, and muscle damage [11,12]. Therefore, the aim of present work is to elucidate the effect of transplanted hematopoietic stem cells on experimentally-induced peptic ulcer in rats.

Material and Methods

Animals: This study was carried out on 30 adult male albino rats (1.50-200 g) at May, 2011. Rats were purchased from the military animals farm (Cairo). The animals were acclimatized in the animal house of the Faculty of Medicine, Suez Canal University for 7 days before the experiment. Rats were housed in fully ventilated cages, with free access to water and food. Animals were divided into 3 groups (n=10 each): 1- Control-rats, each rat was supplemented by single oral dose of normal saline 0.9% (vehicle of ethanol) via an intragastric tube for 4 weeks. 2- Peptic ulcer-non-treated rats, peptic ulcer was induced experimentally by ingestion of one ml of ethanol via intragastric tube [13] and 3- Peptic ulcer-stem cell-treated rats, ethanol-induced peptic ulcer was done by the same way mentioned before. Then a single dose of CD34 positive cells (in a dose of 2x106 cells/rat) was injected intravenously in the rat tail vein [14] after 48 hour of induction of peptic ulcer.

Chemicals: Isolation buffer: Which consists of 100 ml Phosphate buffered saline (PBS) which consists of (0.0 1 M phosphate buffered saline; NaCl 0.138M; KCl-0.0027M) + 0.1% human serum albumin (HSA) + 0.02% (NaN3) and 0.02% (NaN3) and DETACHaBEAD CD34 is supplied as a suspension of 4 x 108 beads/ml in phosphate buffered saline (PBS), pH 7.4, containing 0.1% human serum albumin (HSA) and 0.02% (NaN3) and DETACHaBEAD CD34 is affinity purified polyclonal antibodies. It is supplied sterile filtered in PBS, pH 7.4. (invitrogen, dannel, norway). Dynal CD34 progenitor cell selection system (invitrogen, dannel, norway) which consists of: Dynabeads M-450 MicroMag (SIGMA, DANEL, NORWAY). Dyna beads M-450 CD34 are supplied as a suspension of 4 x 108 beads/ml in phosphate buffered saline (PBS), pH 7.4, containing 0.1% human serum albumin (HSA) and 0.02% (NaN3) and DETACHaBEAD CD34 is affinity purified polyclonal antibodies. It is supplied sterile filtered in PBS, pH 7.4. (invitrogen, danel, norway).

Histopathological and immunohistochemical studies: After collection of gastric juice, the stomach was removed, opened along the greater curvature and washed with saline. The mucosal surface of the gastric body was grossly examined for any changes. The body of the stomach was cut into halves, placed in 10% neutral buffered formalin solution, then processed to prepare 4 paraffin sections suitable for performance of histological Hematoxline & Eosine (H&E) [16] and immunohistochemical techniques using Vimentin anti-human antibody was used in paraffin sections to localize any tissue of human origin in the stomach, using the labeled streptavidin biotin technique. The primary antibody was the Monoclonal mouse Vimentin Anti-human (Dako North America USA).

Isolation of CD34+ stem cells: Umbilical cord blood was collected from normal volunteers prior to expulsion of the placenta while it was still in utero [17]. Separation of stem cells was carried out according to immuno-magnetic separation technique [18]. The quantity of isolated cells were assessed by automatic cell counters. The quality of the isolated CD34+ cells were determined by using trypan blue dye exclusion test, the viable cells were not stained [19].

Equipments: Magnetic separation device (magnetic particle concentrator (DYNAL MPC-I)). (invitrogen, danel, norway). Centrifuge (narco-biosystem, england, U.K.). Spectrophotometer (shimadzu/ double beam Spectro-photometer U.V. 150, Germany). Harvard water bath (narco-biosystem, england, U.K.). Dynal CD34 progenitor cell selection system (invitrogen, danel, norway) which consists of: Dynabeads M-450 CD34 are supplied as a suspension of 4 x 108 beads/ml in phosphate buffered saline (PBS), pH 7.4, containing 0.1% human serum albumin (HSA) of NaOH to endpoint 7.0 and acid content was expressed as $\text{Eq H}^+$ [15].

Statistical analysis: It was performed by Kruskal Wallis one-way ANOVA for multiple comparisons followed by fisher’s PLSD test. Values are expressed as Mean±SD. Post-hoc Scheffe test was applied to identify the source of statistical significance. $p$-values >0.05 were considered statistically significant.
**Results**

Results of the present investigation revealed that peptic ulcer causes statistic ally significant increase \((p < 0.01)\) in the volume of gastric juice when compared to the corresponding value in normal rats. Interestingly, in peptic ulcer-stem cell-treated rats the gastric juice was statistically significantly improved \((p < 0.01)\) lower than the corresponding value in peptic ulcer-non-treated rats as shown in Fig. (1). Also, in the same figure the mean value of free acidity in peptic ulcer-non treated rats was statistically significantly \((p < 0.01)\) higher than the corresponding value in normal control rats. Notably, in peptic ulcer stem cells treated rats, the mean value of free acidity was significantly \((p < 0.01)\) lower than the corresponding value in peptic ulcer-non treated rats. In addition, the mean value of total acidity in peptic ulcer-non-treated group was statistically significantly \((p < 0.01)\) higher than the corresponding value in the normal control rats. Furthermore, in peptic ulcer stem cell-treated rats, the mean value of total acidity was statistically significantly \((p < 0.01)\) lower than the corresponding value in peptic ulcer-non-treated rats. Fig. (2) shows the number of parietal cells/HPF in stomachs of all study groups and the percentage total length of damaged mucosa in all study groups. As the figure shows, the mean value of number of parietal cells/high power field (HPF) which in peptic ulcer non-treated rats was statistically significantly \((p < 0.01)\) higher than the corresponding value in the normal control rats. Interestingly, in peptic ulcer-stem cell-treated rats the mean value of the number of parietal cells/HPF was statistically significantly \((p < 0.01)\) lower than the corresponding mean value in peptic ulcer-non-treated rats. Also, the histogram of Hematoxylin & Eosin stained section for architecture of gastric mucosa revealed that the mean value the percentage of the total length of the damaged mucosa which in peptic ulcer-non treated rats was statistically significantly \((p < 0.01)\) higher than corresponding mean value in the normal control rats. Interestingly, in peptic ulcer-stem cell-treated rats, the mean value of the percentage of the total length of the damaged mucosa was statistically significantly \((p < 0.01)\) lower than the corresponding value in peptic ulcer-non-treated rats Fig. (3) shows a photomicrograph of a stomach section from the control rats revealed normal architecture of the gastric mucosa. Themucosa was formed of epithelium, lamina propria and muscularis mucosa. The epithelium included the surface mucus cells, which were covering the surface and extending down into the gastric pits, and gastric glands. The surface mucous cells appeared as a single layer of tall columnar cells with pale apical cytoplasm and oval basal nuclei. The gastric glands appeared narrow, straight, and perpendicular to the surface epithelium. The gland was formed of the isthmus region which was dominated with surface mucous cells, neck region which was dominated with both mucous neck cells (cubical cells with light basophilic cytoplasm and oval to round basal nuclei) and parietal cells (rounded cells with acidophilic cytoplasm and rounded nuclei and the base region with its chief cells (columnar cells with pale apical cytoplasm and deeply stained basal basophilic cytoplasm surrounding rounded vesicular nuclei) and occasional parietal cells. Also, immunohistochemical stained sections, from the normal control rats showed negative staining for the Monoclonal mouse Vimentin Anti-human. Fig. (4) shows a photomicrograph of a stomach section from peptic ulcer-non treated rat revealed the luminal surface of the gastric mucosa showed superficial or deep erosions with the remnants of the gastric glands and exfoliated cells appearing in the lumen. In uneroded areas, some regions showed marked damage with degeneration or necrosis of most of the cells in the gastric gland. Some of the cells appeared vacuolated with pyknotic nuclei or even karyolitic nuclei. The affection was both in the upper part of the gland and in the base. In some regions, there was complete damage of the cells and distorted shape of gastric glands, lamina propria showed congested blood vessels and inflammatory cell infiltration. Also, immunohistochemical stained sections, from the peptic ulcer-non treated rats showed negative staining for the Monoclonal mouse Vimentin Anti-human. Fig. (5) shows a photomicrograph of a stomach section from the peptic ulcer-stem cells- treated rats showed a picture nearly similar to that of the control group. Most of the luminal surface of the mucosa was intact. The lamina Propria was also nearly similar to normal control rats. An excellent result that; in peptic ulcer-stem cell-treated group the immunohistochemical stained sections, from this rats showed positive brownish staining for the Monoclonal mouse Vimentin Anti-human. The staining appeared granular in the cytoplasm of some cells especially the parietal cells and was observed in some parts of the interstitium (lamina propria).
Fig. (1): A- Histogram showing volume of gastric juice. B- Histogram showing the free acidity of gastric juice and C- Histogram showing the total acidity of gastric juice in (the normal control group, peptic ulcer-non-treated group and peptic ulcer-stem cell-treated group). (N=10 rats in each group). * Significant when compared with the normal control group.
# Significant when compared with peptic ulcer-non-treated group. p-value >0.05 is significant.

Fig. (2): A- Histogram showing the number of parietal cells/HPF in the stomachs. B- Histogram showing the percentage total length of damaged mucosa in (the normal control group, peptic ulcer-non-treated group and peptic ulcer-stem cell-treated group). (N=10 rats in each group) * Significant when compared with the normal control group. # Significant when compared with peptic ulcer-non-treated group. p-value >0.05 is significant.

Fig. (3): A- A photomicrograph of a stomach section from control rats showing the upper part of gastric gland with gastric pits (GP), surface mucous cells arrows, mucous neck cells (M) and parietal cells (P) B- A photomicrograph of a stomach section from control rat showing the lower part of the gastric gland. It shows parietal (P) cells and chief cell (C). Muscularis mucosa (MM) (H&E x400) and C- A photomicrograph of a stomach section from control rat showing negative immunostaining for the Monoclonal mouse Vimentin Antihuman antibody.
Discussion

Ingestion of one ml of ethanol/rat via intragastric tube cause peptic ulcer with significant rise of volume of gastric juice, free acidity and total acidity. These results are caused by direct effect of ethanol which makes glandular stomach covered by hemorrhagic erosion and ulcers with subsequent increase of volume of gastric juice, free acidity and total acidity as seen in the results of the present investigation. Several factors can demonstrate how ethanol cause peptic ulcer through reduction of gastric mucus production, increase free radicals formation, increase acid back diffusion, decrease gastric motility, decrease transmucosal potential difference, decrease endogenous glutathione GSH release, increase 5-Hydroxytryptamine release, increase histamine release, increase sodium and potassium efflux, increase calcium influx, decrease prostaglandin production and decrease mucosal blood flow. Also, Lakshmi et al., [20] concluded that ethanol damages the superficial epithelial...
layers through inhibition of the release of mucosal prostaglandins and depresses its gastric defensive mechanisms. Recently [6], concluded that oxygen derived free radicals produced by ethanol that lead to oxidative damage of mucosa through lipid peroxidation and decrease blood flow, that was reported to be the most accepted mechanisms mediating ulcerogenic effect of ethanol. These results were in agreement with [21-23] That biochemical abnormalities appeared within 1 hour following the ingestion of 1ml of ethanol/rat. But rats exhibited hemorrhagic erosion and ulcer after 1-3min. In addition, Arafa and Sayed-Ahmed [24] concluded that ethanol intake has been shown to be associated with marked oxidative damage to gastric mucosa. The cytoprotective role of antioxidants in the prevention and healing of gastric lesions has been widely investigated in a number of studies. Application of absolute ethanol by gastric gavage induced marked damage to the gastric mucosa and apparent severe oxidative stress in gastric tissue manifested as ethanol stimulate lipid peroxidation via increasing methylene-dioxyamphetamine (MDA) content and reduction of gastric GSH content.

Repetto and Liesuy [25] stated that mucosal damage can be easily produced by the generation of exogenous and endogenous active oxygen and free radicals. Ethanol is involved in the formation of free radicals generated extracellularly and/or intracellularly. Because intragastric administration of superoxide dismutase was able to protect the gastric mucosa against the damaging effect of ethanol, this would suggest the involvement of superoxide free radicals in the pathogenesis of ethanol-induced gastric mucosal damage. Gwinner and Gröne [26] concluded that oxidation of lipids generates lipid radicals which can, in turn, initiate and self-sustain lipid oxidation. Thus, cell and basement membranes that depend on the integration of non-oxidized lipids to maintain their orderly architecture may be deranged, a process that could be important for ulcer formation. Oxidative modification of protein residues can promote the loss of the scaffolding property of structural proteins and can inactivate enzymes. Araki et al., [27] postulate another hypothesis proposed to explain the ethanol-induced oxidative damage to the gastric mucosa is the constrictive effect on veins and arteries of the gastric mucosa, producing congestion, inflammation and tissue injury. Another explanation by Darbar [6], have found that some noxious agents attacking the gastro duodenal mucosa of the host and disturb its integrity which is maintained by an intricate system that provides mucosal defense and repair. Mucus bicarbonate layer form an intricate biologic system, surface epithelial cells and a rich sub mucosal micro-circulatory bed which provides bicarbonate ions which neutralize the acid generated by parietal cell section (HCl), during removing toxic metabolite, the adequate supply of micronutrients and oxygen is supplied by microcirculatory bed so destruction of mucosa will lead to vicious circle of increase gastric juice volume and acidity. The histopathological results, as seen with H&E stain explain the previous data as there was the luminal surface of the gastric mucosa showed superficial or deep erosions with the remnants of the gastric glands and exfoliated cells appearing in the lumen. The percentage of the total length of damaged mucosa was statistically significant, compared to normal control group. In uneroded areas, some regions showed marked damage with degeneration or necrosis of most of the cells in the gastric gland. Some of the cells appeared vacuolated with pyknotic nuclei or even karyolytic nuclei. The affection was both in the upper part of the gland and in the base. In some regions, there was complete damage of the cells and distorted shape of gastric glands. There was also marked increase in the number of parietal cell. The lamina propria showed congested blood vessels and inflammatory cell infiltration these result were in agreement with [6-28,29]. Immunohistochemical stained sections; from peptic ulcer non treated group showed negative staining for the Monoclonal mouse Vimentin Anti-human antibodies. These results were in agreement with [7,30].

In the present investigation, single intravenous administration of haematopoietic stem cells to peptic ulcer rats (in a dose of 2 x 106/rat) caused significant decrease of volume of gastric juice, free acidity, total acidity when compared to the corresponding values in peptic ulcer- non-treated group, in addition H&E stained sections from this group showed a picture nearly similar to that of the normal control group. Most of the luminal surface of the mucosa was intact. The percentage of total length of damaged mucosa was significantly decreased, compared to non treated group. The lamina propria was also nearly similar to normal group. The mean number of parietal cells per high power field which were statistically significant compared to non-treated group. An excellent result that; in peptic ulcer-stem cell-treated group that immunohistochemical stained sections, from this group showed positive brownish staining for the Monoclonal mouse Vimentin Anti-human. The staining appeared granular in the cytoplasm of...
some cells especially the parietal cells and was observed in some parts of the interstitium (lamina propria). These results were in agreement with [31,30] found that both epithelialization and angiogenesis are indispensable processes in gastric ulcer healing that revealed that stem cells secrete high concentrations of growth factors such as vascular endothelial growth factor (VEGF) stimulates expression of antiapoptotic proteins such as Bcl-2 and A 1 in these cells, TGF-beta, and hepatocyte growth factor (HGF) that participate in the healing process of gastric ulcer [32]. Found that angiogenesis is an important event for gastric ulcer healing. VEGF is known to be a potent stimulator of angiogenesis. Bone marrow-derived MSCs expressed mRNAs for certain angiogenic factors (VEGF and HGF), and secreted more VEGF than bone marrow cells in vitro. A majority of the transplanted MSCs maintained their phenotype to produce VEGF and HGF. The results suggest that local injection of MSCs promotes healing of ulcers. Beyth et al., [33] stated that MSCs accelerated the proliferation and migration of residual epithelial cells over denuded areas by releasing TGF-beta, EGF, FGF, and various inflammatory cytokines. In response to deep tissue injury, MSCs proliferate to form a new basement membrane over the epithelial cells and then proliferate and migrate to repair all epithelial tissues. It has also been demonstrated that MSCs have the ability to non-specifically modulate the immune response through their antigen-presenting abilities and through the suppression of dendritic cell. Okamoto et al., [34] have shown damaged epithelia to be regenerated by bone marrow-derived cells in the human GI tract. However, Wagers et al., [35] have shown that both epithelialization and angiogenesis are indispensable processes in gastric ulcer healing. Trans-differentiation of circulating HSCs and/or their progeny is an important event. The histopathological results confirmed the previous data as the percentages of affection (superficial or deep erosions with the remnants of the gastric glands and exfoliated cells appearing in the lumen) significantly decreased when compared to peptic ulcer non-treated group. In the present investigation, Immunohistochemical stained sections, from peptic ulcer stem cell- treated group showed a positive brownish staining for the Monoclonal mouse vimentine Anti-human CD45. Dekel et al., [36], reported that human CD45 represents a universal marker for hematopoietic differentiation therefore it is useful for monitoring CD34 cell engraftment, thus it is used in our study. They even emphasized that their detection correlates with a highly sensitive polymerase chain reaction that detects human-specific Alu sequence (Alu PCR).

References


