ORIGINAL ARICLE

The Therapeutic Value of Erythropoietin in Preventing the Negative Effects of Methotrexate on the Stomach

Ali Hassan A. Ali[1,2], Mohammed Ibrahim Alsuwayl[3], Alaa Hussain Alzuwayyid[3], Mohammed Hamad Aldosari[3], Abdullah Saleh M. Alodaib[3], Talal Hani Arab[3], Mohammed Alqahtani[3], Abdulaziz Fahad Alamer[3], Saleh Abdullah Alkanaan[3], Jaza Alharbi[3], Abdulrahman Saud Abaalhasan[3], Nasser Hassan Al-Swedan[3], Meshari Khalid Alhumaydani[3], Abdulelah Mohammed Alfawaz[3], Tareq A. Althubiti[4]

[1]Department of Anatomy, College of Medicine, Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia, [2]Department of Anatomy, Faculty of Medicine, Al-Azhar University, Cairo, Egypt, [3]College of Medicine, Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia, [4]College of Medicine, Vision colleges, Riyadh, Saudi Arabia

Abstract

Aim: The current study aimed to evaluate any potential protective effects of erythropoietin (Epo) and identify potential histological adverse changes in the stomach caused by methotrexate (MTX) therapy. **Materials and Methods:** There were 60 mature albino rats (about 250 g) in the study. Three equal groups of 20 rats each were created out of the animals. For 9 weeks, the animals in the first control group received intraperitoneal injections of normal saline twice a week at a dose of 0.5 mg/kg. For duration of 9 weeks, animals in Group 2 received intraperitoneal injections of MTX hydrate twice a week at a dose of 0.5 mg/kg. The animals in Group 3 were given intraperitoneal injections of MTX hydrate at a dosage of 0.5 mg/kg twice a week for 9 weeks, along with subcutaneous injections of 100 IU/kg recombinant human Epo once a week. **Results and Discussion:** The stomach tissue's morphological and histological alterations were evaluated. The contents of glutathione peroxidase, superoxide dismutase, glutathione, and IL-2 and IL-6 (interleukin 6) in the serum sample were then determined. Epo demonstrated pronounced antioxidant properties and mitigated MTX's deleterious effects on stomach histology. **Conclusion:** Our findings point to the need for more investigation into the potential use of Epo as a medication to shield patients from MTX's side effects.

Key words: Cytoprotection, erythropoietin, methotrexate, oxidative stress, stomach

INTRODUCTION

The primary organs responsible for synthesizing erythropoietin (Epo), a glycoprotein involved in the regulation of red blood cell synthesis, are the kidneys in adults and the hepatocytes in fetuses.[1] However, it has been demonstrated that other organs, such as the testis, heart, spleen, bone marrow, or brain, express Epo mRNA.[2] The protective roles of blood flow of gastric mucosa, oxygen delivery, and radical oxygen suppression have been the focus of studies on gastric mucosal defense factors. The significance of ferrous ions in the stomach mucosa has also been shown.[3] The direct effects of Epo on the stomach mucosa, however, have not yet been thoroughly studied. Bovine pulmonary artery endothelial cells and human umbilical vein endothelial cells have been used in experiments to examine the effects of recombinant human Epo (rhEpo) on cellular proliferation.[4] These actions may ameliorate the gastric lesions associated with dialysis patients by means of mucosal tissue neovascularization.

Address for correspondence:
Ali Hassan A. Ali, Department of Anatomy, College of Medicine, Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia.
Mobile: 00966560013737/00966115886171.
E-mail: alihassan3750@yahoo.com

Received: 09-03-2024
Revised: 14-06-2024
Accepted: 26-06-2024

The negative effects that patients using methotrexate (MTX) treatment experience affect many important organs. MTX is used in the treatment of several neoplastic conditions, including prostate cancer, non-Hodgkin's lymphoma, breast cancer, and acute lymphoblastic leukemia.[5] MTX is an anti-metabolite drug that is commonly used to treat rheumatoid arthritis and psoriasis, among other conditions. Nonetheless, a major risk is MTX's adverse effects on developing organs.[6] The cytotoxic action of MTX is not exclusive to cancer cells; it also affects healthy tissues with high proliferative activity, such as the alimentary system's mucous membrane and the bone marrow's hematopoietic cells.[7] One well-known complication linked to various standard-dose chemotherapy regimens that are typically used with MTX is gastrointestinal mucositis. The most common sites of mucositis seem to be the mouth and small intestine. According to certain research, MTX damaged the small intestine's mucosa, which resulted in GI ulcers, reduced absorption, nausea, vomiting, diarrhea, and stomatitis. Intestinal mucositis affects about 40% of people using MTX at conventional dosages; at high doses, this percentage has been reported to reach nearly 100%. After intestinal mucositis developed, there is less mucin in the slime layer and it is depleted.[8] Several studies have reported the cytoprotective characteristics of Epo in the heart, kidney, brain, and retina, among other organs, and they have also raised the possibility that Epo could be used therapeutically. Still, there is not much information available about the stomach.[9] The current study's goals were to evaluate any potential protective benefits of Epoand to identify any adverse immunohistochemical and histological changes in the stomach caused by MTX therapy.

MATERIALS AND METHODS

The Al-Kharj Ethical Committee of PSA University's rules for the use and care of animals in research was followed in the performance of our work (SCBR-138-2023). We began our research in March 2023 when the Ethical Committee released the IRB.

Sixty mature male albino rats weighing about 250 g each were picked up from the animal house at PSAU in KSA. They were given a standard rat meal, kept in an animal housing with a temperature of 21–22°C, and had unrestricted access to water.

In the animal housing facility of PSA University's laboratory for experimental surgery and surgical research, the rats were housed in a controlled setting. We bought 2.5 mg MTX tablets from Orion Corporation in Espoo, Finland. However Epo (5000 IU) was purchased from Germany, Mannheim city Company called Recormon, a Roche Diagnostics GmbH product.

Conditions are all consistent with typical and customary laboratory conditions. After the animals were allowed to acclimate for 2 weeks, they were split evenly into three groups of twenty rats each. In the first group (G1), the animals assigned to the control one received intraperitoneal injections of normal saline 2 times per week for a total of 9 weeks at a dose of 0.5 mg/kg BW. For a total of 9 weeks, animals in the second group (G2) received intraperitoneal injections of MTX hydrate at a dose of 0.5 mg/kg BW twice a week. For duration of 9 weeks, animals in Group 3 received intraperitoneal injections of MTX hydrate at a dosage of 0.5 mg/kg twice a week in addition to subcutaneous injections of 100 IU/kg rhEpo once a week. Then, ether inhalation anesthesia has been given to the rats. Rats' left ventricles were used to draw blood samples, and the serum was separated by centrifugation at 1000 g for ten minutes. After that, the serum was stored at −20°C until analysis.

Samples of the stomach were taken out right away after the death. About 10% neutral formalin was used to fix the stomach, and it was left at room temperature for 48 h. All experimental groups' fixed stomach tissues were dehydrated with increasing alcohols, cleaned with xylol, and embedded in paraffin wax. Using a diagnostic kit made by ZellBio Company, the activity of glutathione peroxidase (GPx), glutathione (GSH), and superoxide dismutase (SOD) was measured.[10] The findings are presented in terms of SOD activity units (U) per milliliter. The Abcam Company's ELISA kits were utilized to assess the amounts of proinflammatory cytokines, specifically interleukin 2 and 6, in the serum.

Sections were cut at 5 μm and placed on slides coated with polylysine. Hematoxylin and eosin were used to stain the slides after the paraffin wax was removed using xylene and they were rehydrated using a series of graduated alcohols. Version 17 of the SPSS software program was used for data collection and analysis. F-test (ANOVA) analysis of quantitative data was used to compare the various groups. To calculate the correlation between various parameters, Pearson's coefficient was used. Furthermore, *P* values obtained from the least significant difference (LSD) test that were lower than 0.05 were considered significant.

RESULTS

The control group exhibited thick mucosa on the stomach fundus that was muscularis mucosa separated from the submucosa beneath. The lamina propria contains a rich layer of tall, straight gastric glands. Each one has small pits for stomachs in its base, neck, and isthmus.

Large rounded parietal cells and central rounded nuclei, low columnar mucous neck cells with basal flattened nuclei, and tall columnar mucous secreting cells with basal oval nuclei lined the gastric glands [Figure 1].

The stomach glands in Group II (the group receiving MTX) were desquamated, the gastric pits had reduced,

and the surface epithelial cells had shed into the lumen. In the submucosa, there were clogged blood arteries and inflammatory cell infiltration. Some parietal cells had deep acidophilic cytoplasm and darkly pigmented nuclei, whereas other parietal cells showed vacuum-polymerized cytoplasm. Lesions on the mucosa varied in severity in different sections.

In certain areas, there was an obvious widening between the fundic glands and many sites of tissue loss. Severe dilated blood vessels were seen in the lamina propria and in the crevices between fundic gland cells. On the other hand, the lining epithelium of the gland's basal region was thinner and dilated. The main cells displayed different morphologies; some had flat, highly colored nuclei, while others had cytoplasm that was frothy and vacuolated.

The basal region had many atrophied glands. Mononuclear cellular infiltration was evident in several areas of the lamina propria. Others showed lamina propria masses of lymphoid cells shaped like follicles that reached the base of the fundic gland [Figure 2].

The third group, which was administered Epo, had stomach sections examined, and it was found that the fundus's histological structure was nearly identical to that of the control group.

The fundic glands were in the proper location, and the mucosa seemed to be in good condition.

The fundic gland's lining cells were surface columnar cells with normal-appearing nuclei and cytoplasm [Figure 3]. The MTX group's serum concentrations of the enzyme SOD were considerably lower than those of the control group as shown in Table 1. In addition; Table 2 shows that serum IL-6 levels were considerably higher in the MTX group and much lower in the control group. However, Epo treatment significantly decreased the increase in IL-6. When compared to the MTX group, the control group's blood IL-2 level was statistically significant at 279.68 ± 2.72, according to the data analysis.

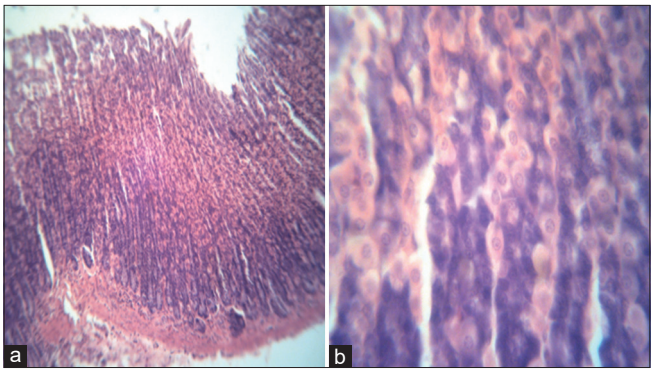

Figure 1: Under a microscope, a section of the stomach belonging to the control group is seen to have several straight, tubular glands that are perpendicular to the surface. (a) H&E, ×200. (b) H&E, ×400

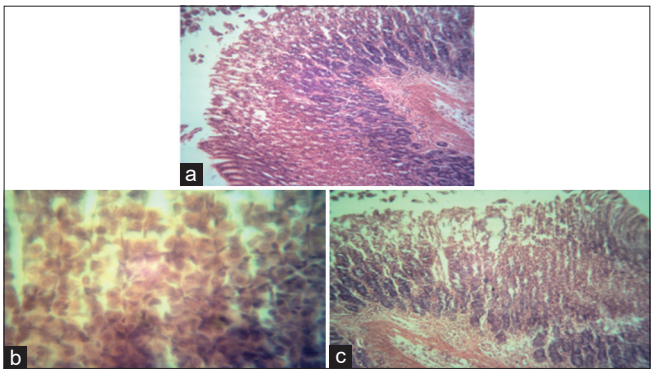

Figure 2: A photomicrograph of a section of the fundic mucosa in the stomach of the MTX-treated group shows many areas of increasing tissue loss in between the fundic gland. Some parietal cells have vacuolization of the cytoplasm. (a) H&E, ×200. (b) H&E, ×400 (c) H&E, ×200

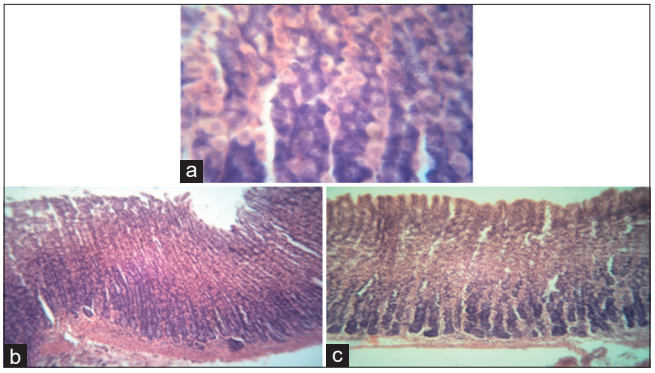

Figure 3: A picture of the glandular stomach mucosa in the group receiving Epo treatment shows that it is almost normal. The fundic glands are in the proper location, and the mucosa seemed intact. (a) H&E, ×400. (b) H&E, ×200 (c) H&E, ×200

DISCUSSION

We investigated the possibility of reducing the damage to stomach tissue resulting from MTX treatment by administering Epo. We saw a significant expansion of the stomach glands in the MTX group compared to the control group, as well as erosion and loss of epithelium on the mucosal surface.

These findings are consistent with a number of recent investigations that showed mice given various natural substances in place of pharmaceuticals that damaged the gastric mucosa saw a large rise in gastric mucus.[11] When used for different types of cancer, chemotherapy treatment produces a number of adverse effects, most of which are in the gastrointestinal tract. Morbidity connected to the treatment is caused by these adverse effects. The stomach epithelium has a quicker turnover rate and is more vulnerable to chemotherapy.[12] Previous research indicates that Epo may prevent hemorrhagic gastric lesions in dialysis patients through both localized effects on the stomach mucosa and systemic hematopoietic

Table 1: Serum superoxide dismutase activity (units/milliliter), Serum GPx activity (units/mL), and Serum GSH (nmol/mL) were used to measure the effects of Epo on the MTX-induced decrease in antioxidant serum parameters.

Groups	Superoxide dismutase (U/mL)	Glutathione peroxidase (U/mL)	Glutathione (nmol/mL)
G1 (Control)	56.74±7.22	79.99±12.88	149.49±14.44
G2 (Methotrexate)	14.39±3.43	51.08±6.73	74.88±9.88
G3 (Methotrexate+Epo)	43.87±5.33	69.43±8.12	95.06±12.42

Table 2: Two distinct metrics were used to evaluate the effect of Epo on the cytokine content generated by MTX: serum interleukin 2 (pg/mL) and serum interleukin 6 (pg/mL)

Groups	Serum interleukin 6	Serum interleukin 2
G1 (Control)	332±28.23	279.68±2.72
G2 (Methotrexate)	522.18±76.11	443.49±3.25
G3 (Methotrexate+Epo)	341.15±5.15	248.21±5.56

effects. Proliferation of gastric mucosal epithelial cells was directly and specifically promoted by Epo.[3]

Our findings supported those of other authors who investigated the deleterious effects of 5-fluorouracil therapy administered repeatedly in rat stomachs, which led to abnormalities in both morphology and function and increased susceptibility of the mucosa to acid.[13] We suggest that the use of MTX makes stomach cells more susceptible to oxidative damage. Furthermore, we suggest that the suppression of oxidative stress by Epo might protect the stomach from harm caused by MTX. Numerous transcription factors, including nuclear factor-Kb (NF-B), are activated in response to the production of ROS. This causes a number of reciprocal biological processes to occur, including an increase in the transcription of genes associated with the development of mucositis. These genes encode proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1and IL-6, Cytokines, in particular TNF-and IL-6, are thought to play a significant role in intestinal inflammation.[14] A small quantity of IL-2 has been found to be a crucial growth factor needed to maintain autoimmunity, based on *in vitro* study. Furthermore, a recent clinical trial study by precedes study found that the combination of MTX and IL-2 improved immunological and clinical responses in rheumatoid arthritis patients and that low doses of IL-2 have novel and helpful therapeutic efficacy in these patients.[15] Thus, we assess the levels of IL-2 and IL-6 in the serum of several research cohorts. The results showed that the animal group receiving MTX had significantly higher levels of these proinflammatory cytokines in their serum as compared to normal levels.

CONCLUSION

The present study aimed to assess if Epo could reduce the adverse effects of MTX on the stomach in an animal model. The study's findings showed that Epo can lessen a variety of histological, inflammatory, and biochemical damage types. These findings also point to the need for more investigation into the potential use of Epo as a medication to shield patients from the harmful effects of chemotherapy.

ACKNOWLEDGMENTS

This publication was supported by the Deanship of Scientific Research at Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia. In addition, we thank those who participated and contributed to the study.

INFORMED CONSENT

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data are available upon request from the authors.

ETHICS APPROVAL

All series of steps that were implemented in this study that included animal models were in compliance with Ethics Committee of Prince Sattam bin Abdulaziz University Institutional Review Board (SCBR-138-2023).

REFERENCES

1. Jelkmann W. Erythropoietin after a century of research: Younger than ever. Eur J Haematol 2007;78:183-205.
2. ElHasnaoui-SaadaniR,MarchantD,PichonA,EscoubetB, Pezet M, Hilfiker-Kleiner D, *et al*. Epo deficiency alters cardiac adaptation to chronic hypoxia. Respir Physiol Neurobiol 2013;186:146-54.
3. Itoh K, Sawasaki Y, Takeuchi K, Kato S, Imai N, Kato Y, *et al*. Erythropoietin-induced proliferation of gastric mucosal cells. World J Gastroenterol 2006;12:234-9.
4. Anagnostou A, Liu Z, Steiner M, Chin K, Lee ES, Kessimian N, *et al*. Erythropoietin receptor mRNA

expression in human endothelial cells. Proc Natl Acad Sci U S A 1994;91:3974-8.
5. Jensen SB, Mouridsen HT, Reibel J, Brünner N, Nauntofte B. Adjuvant chemotherapy in breast cancer patients induces temporary salivary gland hypofunction. Oral Oncol 2008;44:162-73.
6. Dadhania VP, Tripathi DN, Vikram A, Ramarao P, Jena GB. Intervention of alpha-lipoic acid ameliorates methotrexate-induced oxidative stress and genotoxicity: A study in rat intestine. Chem Biol Interact 2010;183:85-97.
7. Hersh EM, Wong VG, Henderson ES, Freireich EJ. Hepatotoxic effects of methotrexate. Cancer 1966;19:600-6.
8. Thorpe D, Butler R, Sultani M, Vanhoecke B, Stringer A. Irinotecan-induced mucositis is associated with goblet cell dysregulation and neural cell damage in a tumour bearing DA rat model. Pathol Oncol Res 2020;26: 955-65.
9. Haine L, Yegen CH, Marchant D, Richalet JP, Boncoeur E, Voituron N. Cytoprotective effects of erythropoietin: What about the lung? Biomed Pharmacother 2021;139:111547.
10. Arthur JR, Boyne R. Superoxide dismutase and glutathione peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. Life Sci 1985;36:1569-75.
11. Tarnawski A, Ahluwalia A, Jones MK. Gastric cytoprotection beyond prostaglandins: Cellular and molecular mechanisms of gastroprotective and ulcer healing actions of antacids. Curr Pharm Des 2013;19:126-32.
12. Yáñez JA, Teng XW, Roupe KA, Fariss MW, Davies NM. Chemotherapy induced gastrointestinal toxicity in rats: Involvement of mitochondrial DNA, gastrointestinal permeability and cyclooxygenase -2. J Pharm Pharm Sci 2003;6:308-14.
13. Kotani T, Nakagiri A, Murashima Y, Takeuchi K. Prophylactic effect of lafutidine against the adverse reaction induced in rat stomach by repeated administration of 5-fluorouracil. Inflammopharmacology 2007;15:203-8.
14. Sultani M, Stringer AM, Bowen JM, Gibson RJ. Anti-inflammatory cytokines: Important immunoregulatory factors contributing to chemotherapy-induced gastrointestinal mucositis. Chemother Res Pract 2012;2012:490804.
15. Zhang X, Miao M, Zhang R, Liu X, Zhao X, Shao M, *et al*. Efficacy and safety of low-dose interleukin-2 in combination with methotrexate in patients with active rheumatoid arthritis: A randomized, double-blind, placebo-controlled phase 2 trial. Signal Transduct Target Ther 2022;7:67.

Source of Support: Nil. **Conflicts of Interest:** None declared.