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Keratinocyte Growth Factor (KGF) and Il-10 are Possible Molecular Targets of Rebamipide Healing Activity In Experimental Oral Mucositis Induced by Chemotherapy

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ABSTRACT

Rebamipide is an antiulcer drug that found to be effective in treatment of chemotherapy induced oral mucositis. This study investigates possible role of keratinocyte growth factor (KGF) and interleukin 10 (IL-10) in rebamipide healing activity. Mucositis was induced by single IP injection of 5- fluorouracil (5-FU) (150 mg/kg). A subsets of 5-FU treated rats were treated with rebamipide suspension (30 mg/kg/ two times a day). Mucositis was assessed according to WHO grading system. Malondialdhyde (MDA), superoxide dismutase (SOD), expression of IL-10, myeloperoxidase (MPO) and KGF was also assessed. Oral mucositis incidence was decreased in Rebamipide treated rats compared to 5-FU. Rebamipide induced a significant ($p \leq 0.001$) decrease in oxidative markers induced by 5-FU. Rebamipide significantly inhibited expression of MPO ($p \leq 0.001$) as well as upregulated IL-10 ($p \leq 0.01$) and KGF ($p \leq 0.001$) compared to 5-FU. In conclusion, Rebamipide ameliorated 5-FU induced oral mucositis. The healing effect of rebamipide may be due to IL-10 and KGF upregulation.

Keywords: Rebamipide, Oral Mucositis, IL-10, KGF

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INTRODUCTION

Oral mucositis is a mucosal inflammation expressed as thinning of oral tissues, erythema and ulceration [1]. This type of mucosal injury is a common side effect of cancer therapy [2]. Incidence of mucositis in chemotherapy treated patients is approximately 40%, raised to more than 50% in high-dose chemotherapy protocols [3]. 5-fluorouracil (5-FU) is a pyrimidine analogue used as chemotherapeutic agent for different types of tumors [4]. Incorporation of 5-FU in chemotherapy protocols increase incidence of oral mucositis as high as 25- 40% of treated patients [5]. 5-FU-treatment is associated with upregulation of pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor –α (TNF-α) [6].

Chemotherapy induces direct epithelial cell injury starting with DNA strand breaks concurrently with production of reactive oxygen species (ROS) [7]. ROS initiate different apoptotic pathways through nuclear factor kappa B (NF-κB) which enhances release of IL-1β and TNF-α and interleukin 6 (IL-6) as a result macrophages are activated stimulating Matrix metalloproteinase (MMPs) leading to primary tissues damage [8, 9]. Mucosal tissues at this stage become ulcerated. The mouth flora can then easily invading to submucosa reaching to systemic circulation causing life-threatening sepsis. Invading microorganism to sub mucosa stimulate inflammatory cell infiltration within injured tissue which in turn release more proinflammatory cytokines such as TNF-α and IL-1β [10, 11]. Decreasing levels of anti-inflammatory cytokines such as IL-10 may also augment mucositis induced by radiotherapy or chemotherapy [12, 13].

This stage of mucositis is painful and impairs patient's quality of life. Moreover, mucositis delays the subsequent chemotherapy cycles until healing of mucosa [14]. Healing process is initiated by signaling pathways that target epithelial cells proliferation and differentiation [1]. Keratinocyte growth factor (KGF) is one of the most important paracrine factors that stimulate epithelial cells growth [15]. The recombinant form of KGF has been approved by FDA for healing induction of oral mucositis induced by chemotherapy and radiotherapy [16]. The proposed mechanism of KGF may be due to upregulation of anti-inflammatory cytokines [17] and down regulation of proinflammatory cytokines [18].

Rebamipide is an anti-ulcer drug that acts via induction of endogenous prostaglandins [19]. Rebamipide also processes an anti-inflammatory activity that may be related to its ability to inhibit NF- κB signaling [20,] suppression of release of interleukin-8(IL-8), IL-1, IL-6 and TNF-α, dawnregulation of inflammatory cell infiltration (assayed as myeloperoxidase) [21,22,23], as well as induction of expression of IL-10 [24]. Moreover, Rebamipide has a potential role in decreasing

oxidative stress [25], In addition, PGE_2 (one of the prostaglandin that induced by rebamipide) stimulated KGF production by gingival fibroblasts [26].

Accordingly, Rebamipide with a potential antioxidant and anti-inflammatory activity represents promising molecule for ameliorating oral mucositis. Moreover , some studies refer to the ability of rebamipide to retard incidence of oral mucositis induced by redio or chemotherapy [27, 28, 29]. We suggest that possible efficacy of rebamipide in promoting healing may be associated with induction KGF expression and upregulation of the anti-inflmmatoy cytokines such as IL-10. This study investigates the possible role of rebamipide in retarding oral mucositis incidence and potential molecular targets that may explain rebamipide healing activity (if any) such as scavenging ROS, expression of KGF and IL-10

MATERIALS AND METHOD

Animals:

Adult male wistar rats weighing 100-150 gm were obtained from animal house of National Research Center (Dokki, Giza, Egypt) and housed in a pathogen-free facility in 6 wire mesh plastic cages with Sawdust bedding. All animal were housed under standard laboratory condition, at 25 ±2 ^{o} C, relative humidity of approximately 50% and a 12-hr light: dark cycle. Rats were fed standard diet and filtered water *ad libitum*.

The experiment was performed in accordance with ethical guidelines of internationally accepted principals for laboratory use and care in animal research (Health research extension act of 1985). Also the study protocol followed the Damanhour University (Egypt) guideline for the use and care of animals.

Preparation of rebamipide:

Rebamipide powder was purchased from Sigma (St.Louis, MO). The drug was suspended in 0.2% methylcellulose dissolved in saline [30]. The resulting solution contains 20 mg/ ml. The suspension was then given by oral gavage at a dose of (30 mg/kg/ two times a day) [31].

Induction of experimental oral Mucositis and experimental groups

Thirty male wistar rats were used during the study. Rats were divided into 3 groups (10 rats each) and treated as follow: **The first group (rebamipide group)**: Rats were treated with rebamipide suspension via oral gavage at a dose of (30 mg/kg/ two times a day) from day 1 to day 8 of the experiment. On the 5th day rats of this group were injected with single I.P injection of 5-FU (150 mg/kg) (EMC united pharmaceutical, Cairo, Egypt) then right cheek pouch was scratched with a wire brush to induce mucositis [32] . **The second group (5-FU group):** Rats were

injected orally with 0.2% methylcellulose dissolved in saline from day 1 to day 8 , at the 5th day rats were injected with single I.P injection of 5-FU (150 mg/kg)[32] then right cheek pouch was scratched with a wire brush to induce mucositis. **The third group (Control group)**: Rats were gavaged with 2% methycellulose in saline solution day 1 to day 8 and single I.P injection of saline on the 5th day.

All animals were scarified at the 9th day and blood samples immediately obtained via cardiac puncture and collected into uncoated tubes and allowed to clot at room temperature for 60 min. The samples were then centrifuged (3000 x g, 10 min, 4◦C), and the resultant serum in each supernatant was collected and stored at -20°C until analysis.

Preparation of mucosa homogenates:

Mucosal tissues of the left pouch were desiccated, weighed and kept frozen under -80˚C. 0.25 gm of tissues was used to prepare 10% (W/V) homogenate in phosphate saline buffer centrifuged at 3000 rpm for 10 min at 4◦C. The obtained supernatant was used for biochemical analysis. Aliquots of the derived supernatant then used for measures of protein content using a standard kit (Wokea Medical Supplies, Changchun, China)

Macroscopic analysis of cheek pouch :

Mucositis was graded at the end of treatment by an three independent pharmacologist who was blinded to the treatments according to World Health Organization (WHO) grading system [33] for mucositis as follow:

Grade	Description
0 (none)	None
I (mild)	Oral soreness, erythema
II (moderate)	Oral erythema, ulcers, solid diet tolerated
III (severe)	Oral ulcers, liquid diet only
IV (life-threatening)	Oral alimentation impossible

Assay of oxidative stress in mucosa tissue:

Two markers of oxidative stress were used in this experiment, malondialdehyde (MDA) and Superoxide dismutase SOD. MDA, the end product of lipid peroxidation, was determined in mucosal homogenate via measurement of thiobarbituric acid (TBA) reaction according to method of Satoh (1978) [34] and kits instructions (Biodiagnostic, Giza, Egypt -CAT No.MD2529).

SOD is an important molecule to detoxify superoxide radical into hydrogen peroxide and molecular oxygen. SOD activity in mucosa tissues was measured based in reaction with reduced phenazine methosulfate and molecular oxygen according to the method of Nishikimi *et al.* (1972) [35] and instruction of kits (Biodiagnostic, Giza , Egypt -CAT No.SD 2521).

Assessment of MPO, KGF and IL-10:

MPO, KGF and IL-10 in tissue homogenates were determined using ELISA kits (wekea medical supplies, china). The detection limit was (0.7-20 ng/ml) for MPO, (20-800 ng/L) for KGF and 2.5-50 ng/L for IL-10. To present results in terms of per-gram tissue protein, protein content in the homogenate was assayed as noted above.

Histopathlogical analysis:

Mucosal tissues of right pouch were removed for histopathological examination. Tissues were fixed in 10% formol saline for twenty four hours. The samples were embedded in Paraffin then 4μm sections were stained by hematoxylin & eosin [36] and examined blindly by histopathologist**.**

Statistical analysis:

Data analysis was performed using the Graphpad Prism version 6 (Graphpad softwere, San Diego, CA, USA). Results were expressed as mean ± standard deviation (SD). Statistical significant difference between groups were done using one-way analysis of variance (ANOVA) and Bonferroni's Multiple Comparison Test as a post hoc test. Categorical variable were compared using Fisher's exact test (two-sided). A probability value of $P \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Rebamipide decreases incidence of mucositis:

Treating rats with 5-FU results in sever mucositis (grade 3 WHO grading system) as evident from erythema and ulceration in 67% of rats (fig 1, fig 2A). Clinical data was confirmed with histopathologic examination which revealed hyperkeratosis and acanthosis of the vacuolized cellular epithelial mucosal layer with finger like projections (Fig 2B and C). Rats treated with rebamipide showed moderate mucositis (grade 1 in 23% and grade 2 in 77% of rats , WHO grading system) with much less erythrema compared to 5-FU treated rats with no evidences of ulcers which was confirmed histopathologically while mucosa of Reb treated rats showed mild acanthosis in the prickle cell of the mucosal lining epithelium (Fig 1, Fig 2D & E). Control rats showed normal histological structure of the stratified squamous keratinized epithelium of the lining mucosa with the underlying lamina propria and muscular layer (Fig 1, Fig 2 F & G)

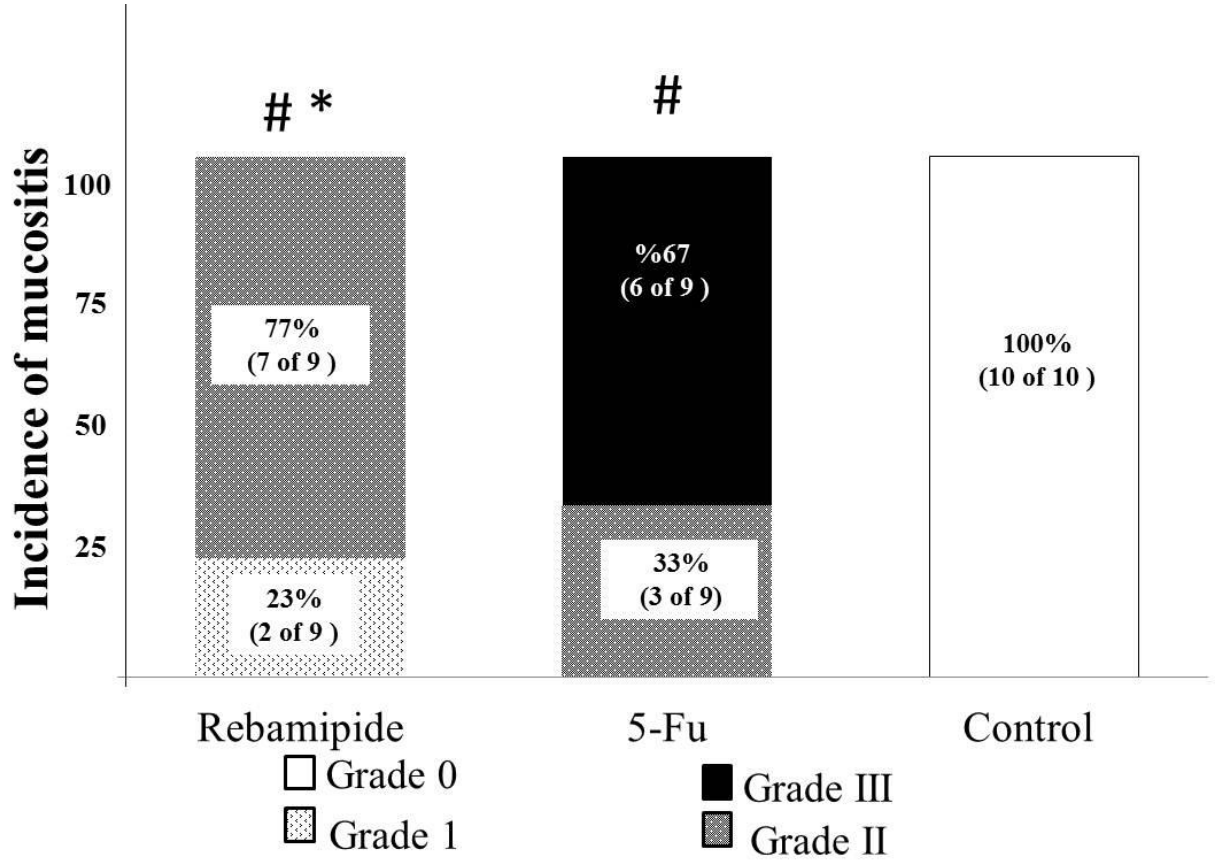

Figure 1: macroscopic analysis of mucosa tissues: Incidence of oral mucositis induced by injection of 5-FU(150 mg/kg) according to WHO grading system. * Significant compared to 5-FU ($p \leq 0.05$), fisher exact test two sided. # significant compared to control rats ($p \leq 0.05$), fisher exact test two sided.

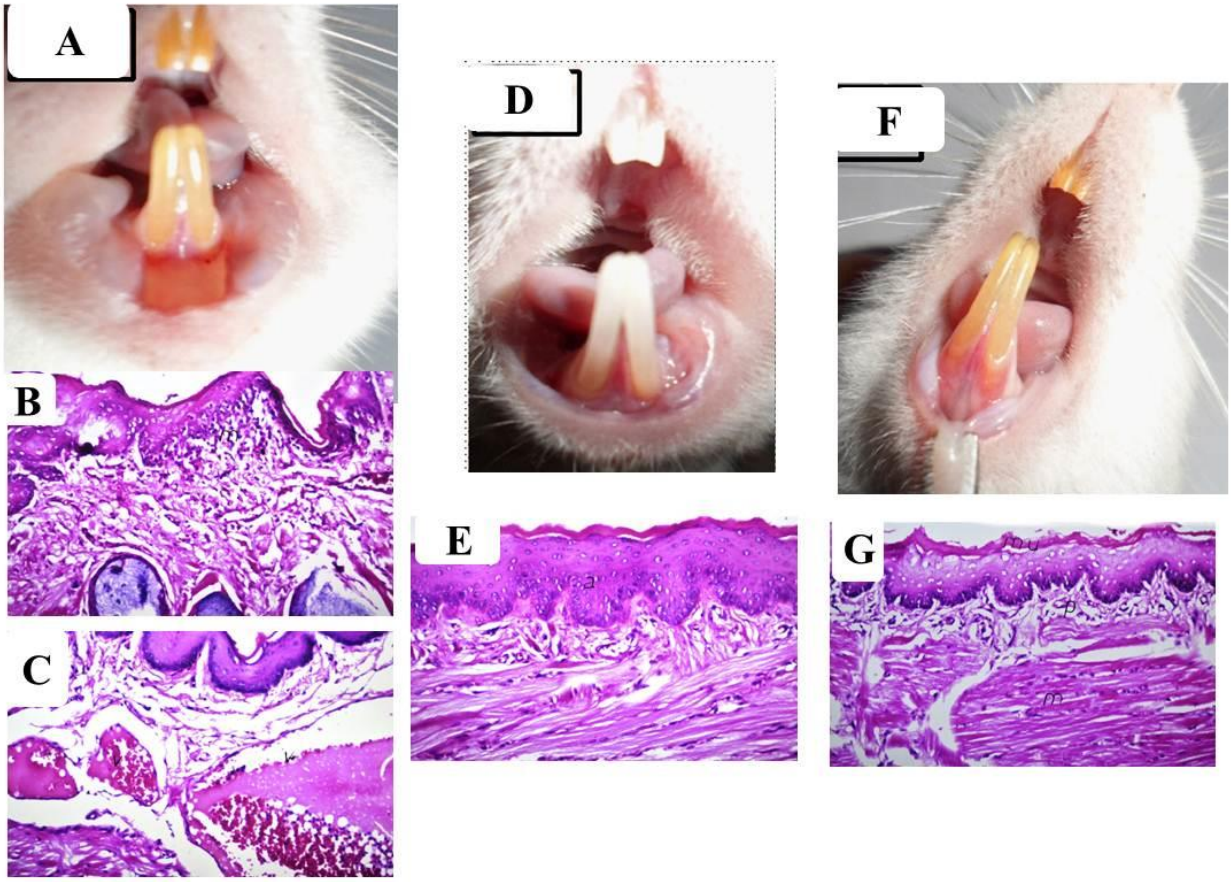

Figure 2: **Macroscopic and histopathlogical analysis of oral mucosa:** Mucosa tissues of the right pouch were processed and stained with Hemtoxylene and Eosin : **(A)** 5-FU treated rats showed erythema and ulceration**. (B and C)** Histologic structure of mucosa from 5-FU treated rats showed hyperkeratosis and acanthosis of the vacuolized cellular epithelial mucosal layer with finger like projections. **(D)** Rebamipide treated rats showed much less erythrema compared to 5-FU with no evidences of ulcers . **(E)** Histological examination of rebamipide treated rats showed mild acanthosis in the prickle cell of the mucosal lining epithelium **(F and G)** Control rats showed normal mucosa and normal histological structure of epithelium of the lining mucosa with the underlying lamina propria and muscular layer.. Images are representative photomicrographs. **Magnification = 40X**.

Effect of rebamipide on mucosal content of antioxidant markers (MDA and SOD): Rats treated with 5-FU showed significant ($p \leq 0.001$) increase (≈ 20%) on mucosa content of MDA (216.6± 21.1) and marked decrease (≈ 65.8 %) in SOD (543.8± 53.6) activity ($p \leq 0.001$) compared to control rats (178.4 ± 15.2) & (1592 ± 121.5) respectively. Rebamipide showed a potent antioxidant effect. It inhibited the formation of MDA (171.1± 9.6) induced by 5-FU

significantly ($p \leq 0.001$). Rebamipide returned MDA to the control level. The drug also prevented the inhibitory effect of 5-FU on SOD (1408±113.3) activity (Fig 3 A, B).

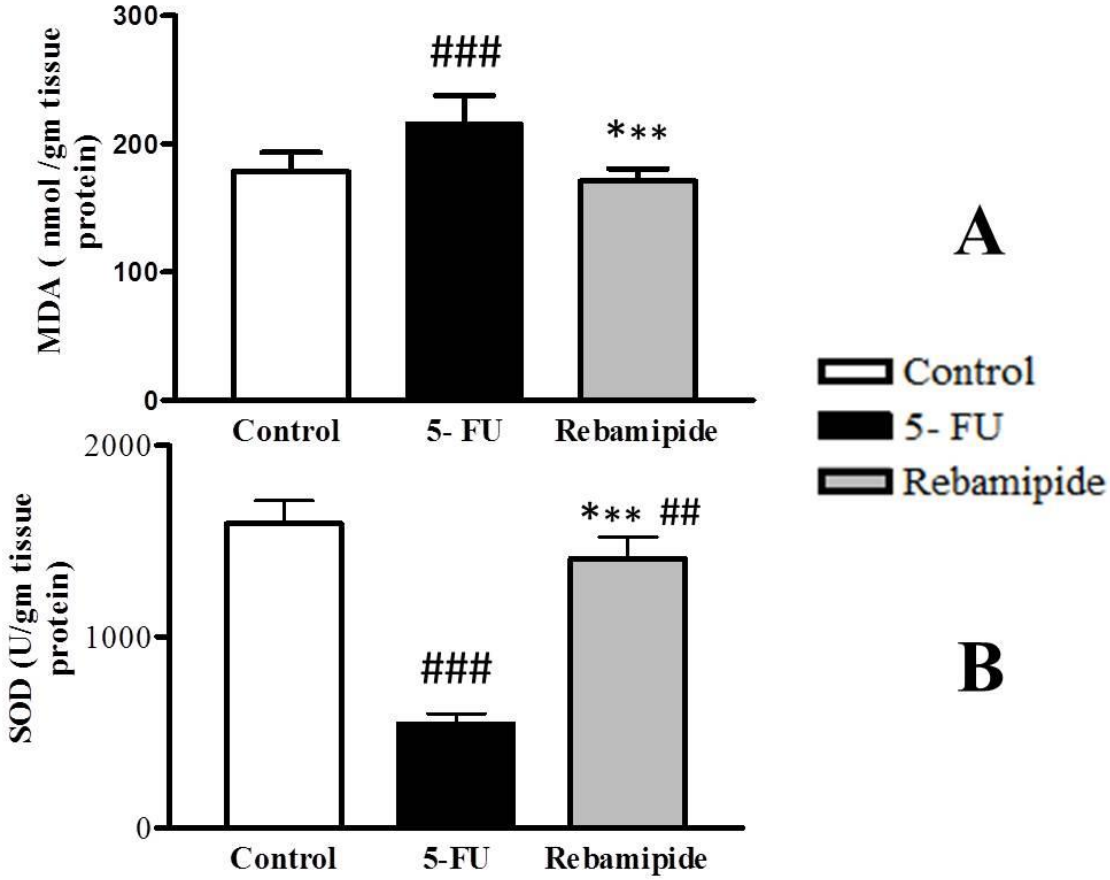

Figure 3: **Effect of rebamipide on mucosa content of antioxidant markers (MDA and SOD): (A)** MDA (in nmol/g tissue protein). **(B)** SOD (in U/g tissue protein). Values shown are means ± SD (n= 10 for control and =9 of other two groups) . Value significantly different compared to control rats $^{\#}$ ($P \leq 0.05$) $^{\#\#}$ ($p \leq 0.01$) $^{\#\#\#}$ ($P \leq 0.001$) or to 5-FU - treated rats * ($P \leq 0.05$) ** ($p \leq 0.01$) ***($p \leq 0.001$).

Anti-inflammatory effect of rebamipide :

Anti-inflammatory effect of rebamipide was evaluated through drug ability to inhibit marker of inflammatory cell infiltration MPO and induction of anti-inflammatory cytokines IL-10. 5-FU increased MPO release in mucosa tissues (1.868± 0.3) significantly ($P \leq 0.001$) compared to control rats (1.103±0.178) in addition, 5-FU inhibited the release of IL-10 (2.35± 0.38) significantly ($p \leq 0.001$) compared to control rats (3.56 ± 0.58). Treating rats with rebamipide significantly ($P \leq 0.001$) attenuated release of MPO (1.262 ± 0.1459) and enhanced release of IL-10 (3.016 ± 0.23) ($p \leq 0.01$) compared to 5-FU respectively. (Fig 4 A, B).

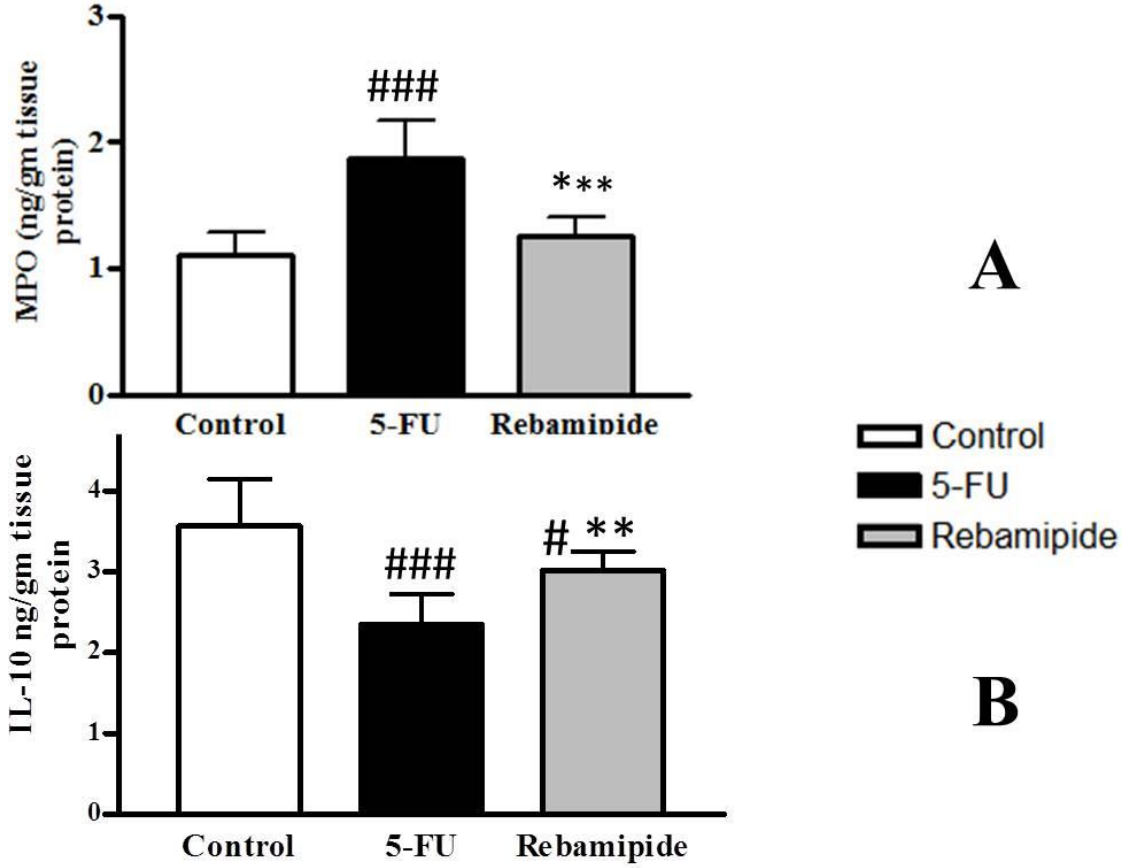

Figure 4: anti-inflammatory Effect of rebamipide : (A) MPO (in ng /g tissue protein). **(B)** IL-10 (in ng /g tissue protein). Values shown are means ± SD (n= 10 for control and =9 of other two groups). Value significantly different compared to control rats $^{\#}$ ($P \leq 0.05$) $^{\#\#}$ ($p \leq 0.01$) $^{\#\#\#}$ ($P \leq 0.001$) or to 5-FU - treated rats * ($P \leq 0.05$) ** ($p \leq 0.01$) ***($p \leq 0.001$).

Healing potential of rebamipide:

The efficacy of rebamipide to induce healing was assessed guided by its effect on KGF. 5- FU significantly ($P \leq 0.001$) decreased expression of KGF (77.96 ± 6.06) compared to control. On the other hand, rebamipide significantly ($P \leq 0.001$) augmented release of KGF (101.1±10.38) in mucosa tissues compared to 5-FU. Rebamipide nearly restored the level of KGF to the normal control level (106.1±10.7). (Fig 5).

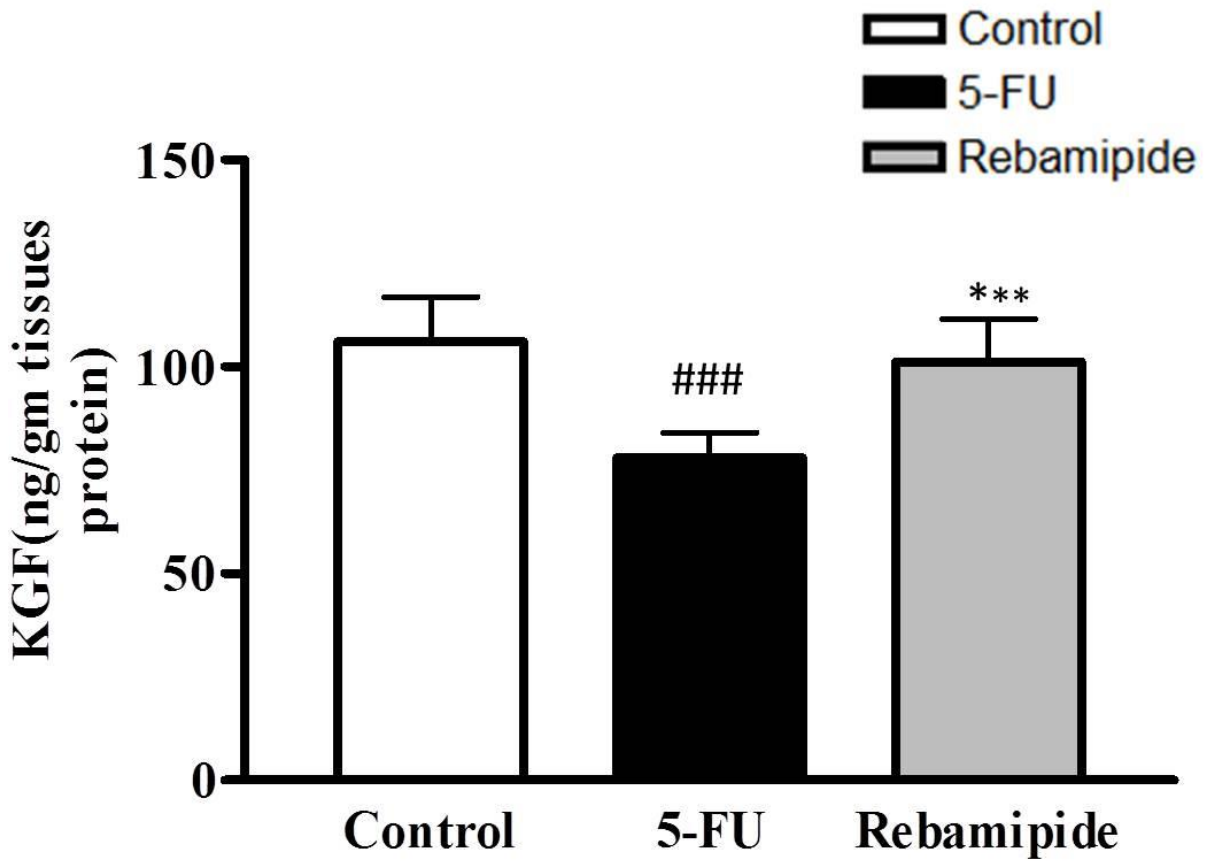

Figure 5: healing potential of rebamipide: KGF (ng/g tissue protein). Values shown are means ± SD (n= 10 for control and =9 of other two groups) . Value significantly different compared to control rats $^{\#}$ ($P \leq 0.05$) $^{\#\#}$ (p≤ 0.01) $^{\#\#\#}$ ($P \leq 0.001$) or to 5-FU - treated rats * ($P \leq 0.05$) ** ($p \leq 0.01$) ***($p \leq 0.001$).

5-FU is highly effective chemotherapeutic agent that is used in treatment of gastrointestinal cancer [37]. However, oral mucositis is a frequent side effect of 5-FU [38]. In general, Chemotherapy induced oral mucosits is associated with generation of ROS especially in early stage of oral mucositis which results in consumption of large quantities of antioxidants molecules including glutathione in the locality of oral mucositis [39]. In addition, proinflammatory cytokines such as (TNF- α, IL-1β, IL-6) are upregulated where anti-inflammatory cytokine such as IL-10 is downregulated in mucosal tissues during mucositis attack [10,11, 12].

Rebamipide is an anti-ulcer drug that induces release endogenous prostaglandin E2 and I2 and exerts a potent free radical scavenging activity [19,27] The drug is also effective as an anti-inflammatory agent. This anti-inflammatory effect is associated with an inhibition of cytokine-mediated neutrophil infiltration, inhibition of reactive oxygen species (ROS) release [31] and

induction of IL-10 [24]. This study evaluated the role of rebamipide in inhibiting oral mucositis incidence and potential molecular targets that may explain this possible healing activity.

This study revealed that rebamipide decreased incidence of 5-FU induced oral mucositis where rat pretreated with rebamipide showed less erythema with no ulceration (moderate mucositis). Different studies reported that Rebamipide gargles has a significant therapeutic efficacy against chemotherapy and radiotherapy induced oral mucositis [27,28]. Moreover, submicronized rebamipide liquid with moderate viscosity has a good healing effect in the rat oral ulcer model [29].

Rebamipide efficacy in preventing oral mucositis may be attributed to its potent antioxidant activity. Our results confirms data from many other studies that reported potent drug efficacy on inhibiting lipid peroxidation and reserving SOD capacity to inactivate superoxide radicals [40, 41 and 42]. In this model of mucositis, rebamipide was able to completely abolish 5-FU effect on lipid peroxidation measured as MDA level as well as it maintain the activity of SOD enzyme in mucosa tissues.

This study showed that rebamipide exerted an anti-inflammatory effect where it inhibited release of MPO as well as increased expression of IL-10. The effect rebamipide on MPO is extensively reported previously in many other studies [30, 23, 43]. Very limited data is available about impact of rebamipide on IL-10 however, in agreement with our results Arakaki et al (2014) [24] who reported that rebamipide increases expression of IL-10 in ocular inflammatory lesion. Similarly, in our pervious study rebamipide oral administration was associated with increased IL-10 in liver tissues [44].

Our results refer to a possible link between rebamipide and KGF mucosa level. Rebamipide significantly abolished 5-FU inhibitory effect on KGF and upregulated IL-10 nearly to the normal control group. There a paucity of data that correlates the healing effect of rebamipide with expression of KGF. However , rebamipide induces prostaglandin E2 which share in induction of KGF[26].

CONCLUSION:

Rebamipide ameliorated 5-FU induced oral mucositis in rats. Rebamipide healing activity was associated with decreased lipid peroxidation and enhancement of ROS scavenging enzymes such as SOD. Moreover, it inhibited inflammatory cell infiltration. Results of this study revealed that rebamipide activity may be in part related to increased expression of IL-10 and healing growth

factors such as KGF however, more studies on KGF receptor expression are needed to point out the relation between rebamipide and KGF.

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REFERENCES:

1. Redding SW, Cancer therapy-related oral mucositis. J Dent Educ 2005; 69 (8) : 919-29.
2. Kolokythas A, Long-term surgical complications in the oral cancer patient: A comprehensive review. Part II J Oral Maxillofac Res 2010; 1(3):e2.
3. Karthaus M, Rosenthal C, Ganser A, Prophylaxis and treatment of chemo- and radiotherapy-induced oral mucositis-are there new strategies? Bone Marrow Transplant 1999; 24 (10):1095-1108.
4. Gamelin E, Boisdron-Celle M. Dose monitoring of 5-fluorouracil in patients with colorectal or head and neck cancer-status of the art. Crit Rev Oncol Hematol 1999; 30: 71-9.
5. De Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, Boni C, Cortes-Funes H, Cervantes A, Freyer G, Papamichael D, Le Bail N, Louvet C, Hendler D, de Braud F, Wilson C, Morvan F, Bonetti A. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol 2000;18(16):2938–2947.
6. Chang C-T, Hsiang C-Y, Ho T-Y, Wu C-Z, Hong H-H, Huang Y-F . Comprehensive Assessment of Host Responses to 5-Fluorouracil-Induced Oral Mucositis through Transcriptomic Analysis. PLoS ONE 2015; 10(8): e0135102.
7. Sonis ST, Oral mucositis in cancer therapy. J Support Oncol 2004;2(6):3-8.
8. Sonis ST, The biologic role for nuclear factor-kappaB in disease and its potential involvement in mucosal injury associated with anti-neoplastic therapy. Crit Rev Oral Biol Med 2002;13(5):380-9
9. Bamba S, Andoh A, Yasui H, Araki Y, Bamba T, Fujiyama Y. Matrix metalloproteinase-3 secretion from human colonic subepithelial myofibroblasts: role of interleukin-17. J Gastroenterol 2003;38:548-54.

10. Sonis ST, Pathobiology of oral mucositis: novel insights and opportunities. J Support Oncol 2007 5 (9):3-11.
11. McCarthy GM, Awde JD, Ghandi H, Vincent M, Kocha WI, Risk factors associated with mucositis in cancer patients receiving 5-fluorouracil. Oral Oncol 1998; 34(6):484-90.
12. Silva GB, Sacono NT, Othon-Leite AF, Mendonça EF, Arantes AM, Bariani C, Duarte LG, Abreu MH, Queiroz-Júnior CM, Silva TA, Batista AC, Effect of low-level laser therapy on inflammatory mediator release during chemotherapy-induced oral mucositis: a randomized preliminary study. Lasers Med Sci 2015;30(1):117-26.
13. Moura JF, Mota JM, Leite CA, Wong DV, Bezerra NP, Brito GA, Lima V, Cunha FQ, Ribeiro RA, A novel model of megavoltage radiation-induced oral mucositis in hamsters: Role of inflammatory cytokines and nitric oxide. Int J Radiat Biol 2015;91(6):500-9.
14. Schmidt E, Thoennissen NH, Rudat A, Bieker R, Schliemann C, Mesters RM, Zühlsdorf M, Müller-Tidow C, Berdel WE. Use of palifermin for the prevention of high-dose methotrexate-induced oral mucositis. Ann Oncol 2008; 19(9):1644-9.
15. Liu CJ, Jin JD, Lv TD, Wu ZZ, Ha XQ. Keratinocyte growth factor gene therapy ameliorates ulcerative colitis in rats. World J Gastroenterol 2011; 17(21):2632-40.
16. Meropol NJ, Somer RA, Gutheil J, Pelley RJ, Modiano MR, Rowinsky EK, Rothenberg ML, Redding SW, Serdar CM, Yao B, Heard R, Rosen LS. Randomized phase I trial of recombinant human keratinocyte growth factor plus chemotherapy: potential role as mucosal protectant. J Clin Oncol 2003; 21(8):1452-8.
17. Krijanovski OI, Hill GR, Cooke KR, Teshima T, Crawford JM, Brinson YS, Ferrara JL. Keratinocyte growth factor separates graft-versus-leukemia effects from graft-versus-host disease. Blood 1999;94(2):825-31
18. Blijlevens N and Sonis S. Palifermin (recombinant keratinocyte growth factor-1): a pleiotropic growth factor withmultiple biological activities in preventing chemotherapy- and radiotherapy-induced mucositis. Ann Oncol 2007; 18(5):817-26.
19. Arakawa T, Kobayashi K, Yoshikawa T, Tarnawski A. Rebamipide: Overview of mechanisms of action and efficacy in mucosal protection and ulcer healing. Dig Dis Sci 1998; 43:5S-13S
20. Li W, Zhao Y, Xu X, et al, Rebamipide suppresses TNF-α mediated inflammation in vitro and attenuates the severity of dermatitis in mice. FEBS J 2015; 282:2317-2326.

21. Choe JY, Park KY, Lee SJ, Park SH, Kim SK. Rebamipide inhibits TNFα-induced IL-8 expression by suppressing the NF-κB signal pathway in human umbilical vein endothelial cells. Inflamm Res 2010; 59:1019-1026.
22. Fukuda K, Ishida W, Tanaka H, Harada Y, Fukushima A. Inhibition by reba-mipide of cytokine-induced or LPS-induced chemokine synthesis in human corneal fibro-blasts. Br J Ophthalmol 2014; 98:1751-1755.
23. Funahashi Y, Yoshida M, Yamamoto T, Majima T, Takai S, Gotoh M. Intravesical application of rebamipide suppresses bladder inflammation in a rat cystitis model. J Urol 2014; 191(4):1147-52.
24. Arakaki R, Eguchi H, Yamada A, et al. Anti-inflammatory effects of rebamipide eye drop administration on ocular lesions in a murine model of primary Sjögren's syndrome. PLoS One 2014; 9: e98390.
25. Hahm KB, Lee KJ, Kim YS, et al. Augmented eradication rates of Helicobacter pylori by new combination therapy with lansoprazole, amoxicillin, and rebamipide. Dig Dis Sci 1998; 43:235-240.
26. Mitsuhiro O, Yoko Y, Kichibee O. Synergistic effects of prostaglandin E1 (PGE1) or PGE2 and dexamethasone on keratinocyte growth factor (KGF) production by gingival fibroblasts. International Congress Series, Volume 1284, Issue null, Pages 221-222
27. Shinohara A, Nakamura M, Onikubo T, Nakamura K. Efficacy of Rebamipide Gargle against Chemotherapy-induced Oral Mucositis. Yakugaku Zasshi 2015; 135(8):937-41.
28. Yasuda T, Chiba H, Satomi T, Matsuo A, Kaneko T, Chikazu D, Miyamatsu H. Preventive effect of rebamipide gargle on chemoradiotherpy-induced oral mucositis in patients with oral cancer: a pilot study. J Oral Maxillofac Res 2012;2(4):e3.
29. Nakashima T, Sako N, Matsuda T, Uematsu N, Sakurai K, Ishida T. Novel submicronized rebamipide liquid with moderate viscosity: significant effects on oral mucositis in animal models. Biol Pharm Bull 2014; 37(4):671-8.
30. Diao L, Mei Q, Xu JM, Liu XC, Hu J, Jin J, Yao Q, Chen ML. Rebamipide suppresses diclofenac-induced intestinal permeability via mitochondrial protection in mice. World J Gastroenterol 2012; 18(10):1059-66.
31. Sakurai K, Osaka T, Yamasaki K. Protection by rebamipide against acetic acid-induced colitis in rats: relationship with its antioxidative activity. Dig Dis Sci 1998;43(9):125S-133S.

32. Gawish SA, Abou-Elez Nosseir DA, Omar NM, Sarha NMR. Histological and Ultra Structural Study of 5-fluorouracil-induced Small Intestinal Mucosal Damage in Rats. Asian Journal of Cell Biology 2013; 8 (1) :1
33. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981; 47(1):207-14.
34. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta 1978; 90(1):37-43.
35. Nishikimi M, Appaji N, Yogi K. The occurrence of superoxide anion in the reaction of reduced phenazinemethosulfate and molecular oxygen. Biochem Bioph Res Commun 1972; 46 (2): 849-854.
36. Banchroft JD, Stevens A , Turner DR. Theory and practice of histological techniques .4th Ed., New York , London , San Francisco , Tokyo: Churchil Livingstone; 1996.
37. Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. Nature Reviews Cancer 2003; 3(5):330–8.
38. Jones JA, Avritscher EB, Cooksley CD, Michelet M, Bekele BN, Elting LS. Epidemiology of treatment-associated mucosal injury after treatment with newer regimens for lymphoma, breast, lung, or colorectal cancer. Support Care Cancer 2006;14 (6):505–15.
39. Yoshino F, Yoshida A, Nakajima A, Wada-Takahashi S, Takahashi S-s, Lee MC-I. Alteration of the Redox State with Reactive Oxygen Species for 5-Fluorouracil-Induced Oral Mucositis in Hamsters. PLoS ONE 2013; 8(12): e82834
40. Park NC, Park HJ, Lee KM, Shin DG. Free radical scavenger effect of rebamipide in sperm processing and cryopreservation. Asian J Androl 2003;5(3):195-201.
41. Nagano Y, Matsui H, Shimokawa O, Hirayama A, Tamura M, Nakamura Y, Kaneko T, Rai K, Indo HP, Majima HJ, Hyodo I. Rebamipide attenuates nonsteroidal anti-inflammatory drugs (NSAID) induced lipid peroxidation by the manganese superoxide dismutase (MnSOD) overexpression in gastrointestinal epithelial cells. J Physiol Pharmacol 2012; 63(2):137-42.
42. Murrell DE, Rahmasari Y, Denham JW, Panus PC, Harirforoosh S. Celecoxib or diclofenac hepatic status in the presence or absence of rebamipide. Eur Rev Med Pharmacol Sci 2015; 19(17):3318-25.

43. Katada K, Yoshida N, Isozaki Y, Tomatsuri N, Ichikawa H, Naito Y, Okanoue T, Yoshikawa T, Prevention by rebamipide of acute reflux esophagitis in rats. Dig Dis Sci 2005;50(1):S97-S103.
44. Zakaria S, El-Sisi A. Rebamipide retards CCl4-induced hepatic fibrosis in rats: Possible role for PGE2. J Immunotoxicol 2016 :1-10.