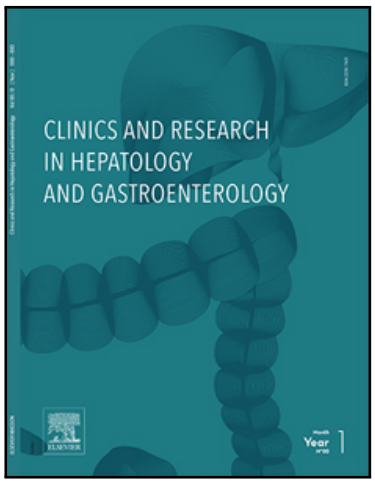

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The role of Zinc L-Carnosine in the prevention and treatment of gastrointestinal mucosal disease in humans: a review.

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REVIEW HIGHLIGHTS

- Zinc L-carnosine is a safe mucoprotective agent used to treat peptic ulcers
- Recent evidence may extend its indications to include proximal or distal GI disease
- Zinc L-carnosine may increase *H. pylori* eradication rates associated with current regimens

- Increased healing rates of post-ESD ulcers suggest a potential use in operative endoscopy
- Efficacy on intestinal permeability maybe useful in functional bowel disorders
- Targeted formulations with increased adhesiveness are needed to increase effectiveness

ABSTRACT

Zinc L-carnosine is a pharmaceutical compound with direct mucosal cytoprotective and anti-inflammatory action through its antioxidative effects, cytokine modulation and membrane-stabilizing properties. Chemically, it is not an anti-secretory, antacid or raft-forming agent; its properties are mainly mediated by its higher affinity for damaged mucosa that permits the release of zinc locally by ligand exchange. Beneficial effects on various types of mucosal damage have been described *in vitro* and *in vivo*, in both animals and humans. It has been shown to promote repair of mucosal injury in human studies and has been widely used for the treatment of peptic ulcers, chemoradiotherapy-induced oral mucositis and esophagitis. More recently, the therapeutic applications of Zinc L-carnosine have been extended to the prevention and cure of various types of intestinal damage, including ulcerative colitis, iatrogenic ulcers after operative endoscopy, hemorrhoidal disease and impaired intestinal permeability. This review concentrates mainly on the current and future applications of zinc L-carnosine in gastrointestinal disease, and may be of use to gastroenterologists and endoscopists. It describes the therapeutic principles and benefits of this interesting molecule and discusses the potential future fields of interest for clinical use in humans.

Keywords: Zinc L-Carnosine; polaprezinc; mucosal damage; gastric ulcer;

mucosal permeability; endoscopic submucosal dissection; ESD

INTRODUCTION

Zinc is a trace metal implicated in various metabolic pathways, with documented roles in health and disease. It is a component of various enzymatic systems, including zinc-dependent matrix metalloproteinases, which play a role in protein and DNA synthesis, cell division and immunity. Its deficiency can lead to various clinical consequences that include failure to thrive, skin rash, and impaired wound healing. Zinc is also an anti-oxidant, with oxygen radical scavenging ability [1, 2]. Oral and topical administration of zinc seems to stimulate the healing of gastric ulcers [3], experimental colonic inflammation [4] and skin wounds [1], even in the absence of deficiency; zinc sulfate has also been suggested for the prevention of radiation-induced oropharyngeal mucositis [5].

L-Carnosine is a dipeptide composed of beta-alanine and L-histidine, mainly but not exclusively present in animal muscle. It is a highly water-soluble buffering agent; it possesses three ionizable groups, including a carboxylic group and an imidazole ring. It is involved in the normal physiological function mainly of excitable tissues (skeletal and cardiac muscle, neurons), with different putative functions within each. It has been studied extensively for its antioxidant activity, mediated by different mechanisms involving metal ion chelation, and scavenging reactive oxygen species and peroxyl radicals. In physiological conditions, carnosine has been found to reduce oxidative damage and to improve the overall enzymatic and non-enzymatic antioxidant activity in the liver, brain, skeletal muscle and kidney. Furthermore, in animal models of disease, L-carnosine has exhibited potential therapeutic effects in controlling glycemia, ischemic damage, wound healing, neoplastic cell proliferation, neurodegeneration, and ageing processes. Data from animal and human studies suggest that carnosine can accelerate wound healing and chemoradiotherapy-induced lung injury [6].

Zinc L-carnosine (beta-alanyl-L-histidinato zinc), also called polaprezinc in the scientific literature, is a chelate compound of zinc and L-carnosine,

in a quadridentate 1:1 complex [7]. Chemically, it is not an anti-secretory, antacid or raft-forming agent, thus does not control acid exposure of the mucosa or luminal electrolyte chemistry; its therapeutic properties are linked to the biological effects of its constituents and to its higher affinity to damaged tissue. It has been used, primarily in Japan, as an anti-ulcer drug for over 2 decades [8]. Recent research has proposed novel therapeutic targets, ranging from *Helicobacter pylori* eradication to oral and gastrointestinal mucosal protection against chemical and inflammatory insult. This review aims to summarize the available evidence on the current and potential gastrointestinal applications of zinc L-carnosine. We focus primarily on human studies, illustrating the main therapeutic areas, benefits and limitations of this molecule, and discuss possible future uses of interest to gastroenterologists and endoscopists.

USE OF ZINC L-CARNOSINE IN MUCOSAL DISEASE

Mechanism of action

Both zinc and carnosine have beneficial effects on mucosal tissues but low local adhesion. Zinc L-carnosine, being insoluble in saline but dissolvable in acid, and polymeric in nature, shows a slow dissociation rate in the stomach, which prolongs the local therapeutic effects of its constituents, without affecting gastric emptying [9]. Furthermore, it shows a higher affinity for the ulcerous site in animal models, both in terms of local adhesiveness [9] and zinc tissue content over time [10]. It is assumed that a ligand exchange reaction takes place, preferentially at the lesion site, binding zinc to other exposed carrier proteins and freeing L-carnosine locally [8]. L-carnosine is metabolized rapidly to L-histidine and b-alanine, not reaching the bloodstream; zinc plasma concentrations increase rapidly after administration, only slightly affecting zincemia at therapeutic dosages [11].

Once bound to the damaged mucosa, zinc L-carnosine exhibits local membrane-stabilizing [12] and antioxidant effects in *in vitro* [13, 14], *ex vivo* [15] and animal models [15]. It scavenges superoxide anion and hydroxyl radicals, inhibits lipid peroxidation [13, 14] and increases the expression of antioxidant enzymes [15], in the gastric and intestinal mucosa. On the other hand, it does not affect acid secretion or prostaglandin E_2 levels in the mucosa when given intragastrically, nor is less effective in the presence of indometacin. This suggests that the mechanism of mucosal protection is not mediated by acid suppression or endogenous prostaglandins [16]. Zinc L-carnosine has been shown to have anti-inflammatory effects by suppressing the overexpression of nuclear factor-kappaB (NF-κB), an inflammatory transcription factor, in macrophages [17, 18] *in vitro*, as well as in murine colonic mucosa [19] and plasma [18], in experimental inflammation models. In rats, it suppressed interleukin (IL)-6, IL-8 and tumor necrosis factor (TNF)-α expression in damaged gastric mucosa [15]. Similarly, zinc L-carnosine attenuated mucosal mRNA expression for IL-1β, IL-2, IL-6, interferon (IFN)-γ and TNF-α in experimental models of murine colitis [20]. Furthermore, it has been shown to stimulate heat shock protein 72 (HSP72), a cytoprotector against protein-damaging cellular stress, in a similar setting [19]. Gene expression of Insulin-like Growth Factor (IGF)-1, a polypeptide that plays an important role in cellular proliferation and differentiation, was also increased after zinc L-carnosine stimulation in cultured endothelial cells or fibroblasts [21] (see Figure 1 for a graphical overview).

A number of studies have indicated that the antioxidant and anti-inflammatory activity of zinc L-carnosine is primarily linked to the zinc component. *In vitro* superoxide inhibition and *in vivo* mucosal HSP72 induction and reduction of colonic inflammation were replicated by zinc sulfate administration, although they proved demonstrably much weaker; L-carnosine alone did not exhibit beneficial effects on either [13, 19]. Similarly, both zinc L-carnosine and zinc sulfate suppressed stimulated IL-2 gene expression *in vitro* [20].

Nevertheless, some evidence exists on the anti-inflammatory properties of carnosine. *In vitro,* TNF-α-induced IL-8 expression in intestinal epithelial cells was suppressed in the presence of carnosine [22], possibly by virtue of its histidine component [23]. In an animal model of experimental portal hypertensive gastropathy, hydrocloric acid injury was reduced by oral zinc sulfate and zinc L-carnosine but not L-carnosine alone. However, both L-carnosine and zinc were independently capable of significantly inducing HSP72 activity in the mucosa in that study, suggesting a joint cytoprotective effect [24].

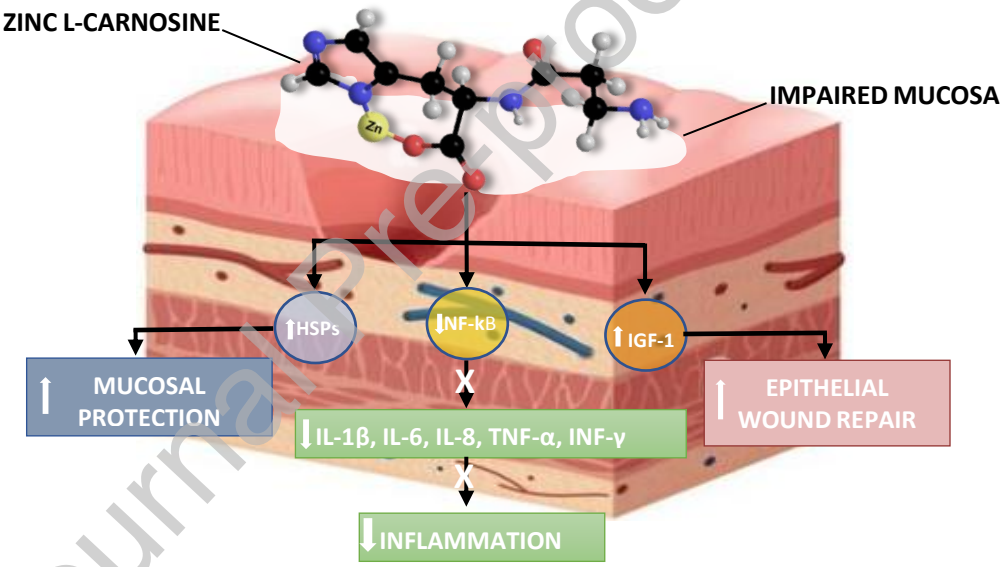

Figure 1. An overview of the proposed cytoprotective, anti-inflammatory and healing mechanisms of zinc L-carnosine.

Pharmacokinetics

In early animal studies utilizing radioactive ^{14}C and ^{65}Zn-labeled compounds, zinc L-carnosine showed markedly higher residence times in the stomach, particularly in the first 1-4 hours after administration, with peaks about 200 times higher than that of L-carnosine and 40 times higher that of zinc sulfate. Furthermore, it demonstrated greater

concentrations in gastric tissues than both its constituents, with ^{14}C- and ^{65}Zn-radioactivity levels on ulcerous sites almost double those of the normal mucosa, suggesting greater mucosal adhesion and not mere accumulation in the lumen, as well as a clear predilection for ulcers [9]. Following zinc L-carnosine administration, $^{65}Zn/^{14}C$ ratios in the gastric tissue remained stable at 30 minutes, followed by a 3-fold increase by minute 60, suggesting dissociation and degradation of L-carnosine, while Zn ions persisted locally. Importantly, when administering labeled zinc L-carnosine in acid solution, and thus partially dissociated, gastric tissue ^{65}Zn and ^{14}C radioactivity was greatly reduced, while $^{65}Zn/^{14}C$ ratios remained constant, suggesting that mucosal adhesion is greater when the compound is intact and then gradually dissociating at the mucosal level by ligand exchange [8, 9]. Animal studies combining zinc L-carnosine administration with acid suppression have further confirmed this by demonstrating significantly higher and more persistent Zn concentrations in the gastric mucosa of rats treated with cimetidine [9].

Plasma ^{14}C and ^{65}Zn radioactivity levels increased differentially, showing a more gradual increase of the ^{14}C component throughout, which peaked at 8 hours from administration, and a somewhat flatter initial curve for ^{65}Zn peaking at 4 hours and followed by rapid decline. ^{14}C and ^{65}Zn curves for L-carnosine and zinc sulfate demonstrated very early peaks (<2 hours) and higher overall levels before final tapering. These observations are consistent with a low absorption rate of zinc L-carnosine, owing to an initial dissociation of the compound in the gastrointestinal tract, followed by separate metabolic pathways for Zn and L-carnosine [9].

Following a single administration of labeled zinc L-carnosine in animal studies, a rapid increase in luminal ^{65}Zn radioactivity was observed in the stomach and duodenum, followed by a later peak in the ileum and caecum [25]. In rats, reported accumulated excretion rates for ^{14}C radioactivity were 38.8% in exhalation, 13.3% in feces and 4.1% in urine, while for ^{65}Zn 85% in feces and 0.3% in urine, with an estimated absorption rate of zinc of approximately 11% at 50 mg/kg [8, 26, 27]. Furthermore, increase of blood ^{65}Zn radioactivity was not dose-

dependent, likely because of saturation of intestinal absorption at high doses of zinc L-carnosine (>30 mg/kg) [26, 27].

Applications in gastrointestinal disease

Overall, zinc L-carnosine is a compound that acts locally and directly on the damaged mucosa, through multiple anti-inflammatory and cytoprotective pathways, showing negligible systemic effects at usual therapeutic dosages (see also the *Safety* section). These characteristics, together with a slow dissociation rate, relative site selectivity and acid-independent mechanism of action, render this compound promising both as a useful add-on to standard regimens (e.g. in conjunction with proton pump inhibitors), and as a stand-alone treatment.

Due to these well documented mechanisms of action and favorable characteristics, several applications of zinc-L-carnosine have been described, both in animal models and human diseases. We summarize the available human data in Table 1.

Peptic ulcers

The ability of zinc L-carnosine to prevent gastric lesions has been extensively studied in animal models. Despite its longstanding use and empiric efficacy in the treatment of peptic ulcer, particularly in Japan, human studies are few and dated.

Zinc L-carnosine has been shown to inhibit gastric ulcer formation after ethanol or indomethacin administration in rats [16]. A recent study on ethanol-induced gastric ulcers in rats confirmed that pretreatment with zinc L-carnosine resulted in significantly lower ulcer indices in a dose-dependent manner. Also, post-ethanol expression of inflammatory cytokines in the gastric mucosa (IL-1β, IL-6, IL-8 and TNF-α) significantly decreased in a dose-dependent manner. This was paralleled by a significant increase in the expression of anti-oxidant enzymes (superoxide dismutase 1, heme oxygenase 1, glutathione and

peroxidredoxin 1), growth factors (nerve growth factor, vascular endothelial growth factor and platelet-derived growth factor B) and cytoprotective heat shock proteins (HSP90, HSP70, HSP60, HSP27, and HSP10) in the mucosa [15].

Initial Japanese studies in the '90s demonstrated the efficacy of zinc L-carnosine in the treatment of gastric ulcers in humans. An early randomized, multi-center, placebo-controlled, double-blind trial on 299 patients with confirmed gastric ulcers randomly assigned, within 1 week of endoscopy, to 150 mg daily zinc L-carnosine or 800 mg of cetraxate hydrochloride (a mucosal protective agent) or placebo for 8 weeks. Symptom improvement (epigastric pain, heartburn, nausea, diarrhea, constipation, hematemesis, melena), reported as "marked", was similar in the treatment groups at 4 and 8 weeks, but superior to placebo. The same was true for the endoscopic healing rate for the zinc-carnosine group and the cetraxate group respectively at 4 weeks (26.3% vs. 16.2%) and at 8 weeks (60.4% vs. 46.2%) [28].

Another double-blind multicenter trial compared the efficacy of zinc-carnosine 50 mg, 75 mg, or 100 mg twice daily. Symptoms and endoscopic healing rates improved in all groups. Similar improvement rates were observed for symptoms and endoscopic cure rates between groups. The optimal dosage appeared to be 75mg twice daily with 24% and 66% of healed ulcers and at least a moderate symptomatic improvement in 84% and 94% of patients, at 4 and 8 weeks, respectively [29]. Slightly better healing rates were reported in subsequent studies, with an endoscopic healing rate of 31.6-37.8% at 4 and 67.4-80.0% 8 weeks, and no significant adverse events [30, 31]. Compared to sucralfate, zinc L-carnosine showed similar efficacy in terms of endoscopically assessed ulcer healing (71.3% vs. 75.5%) [32].

Owing to its unique mechanism of action and site selectivity, zinc L-carnosine can be used in conjunction to current anti-secretory treatments or more importantly may be employed when such treatments need to be avoided or dose-adjusted.

Helicobacter Pylori eradication

H. pylori urease, a buffering agent that facilitates bacterial colonization of the stomach, is inhibited by some bivalent cations, such as Zn^{2+}, possibly because of the substitution of its essential nickel ion [33]. *In vitro* studies have shown that zinc compounds inhibit *H. pylori* growth in a non-reversible manner [34]. In addition, zinc L-carnosine inhibits *H. pylori*-mediated gastric inflammation, as well as bacterial adhesion [35].

In an early human study, evaluating a 7-day amoxicillin 500mg + clarithromycin 400mg + lansoprazole 30mg twice daily regimen with or without zinc L-carnosine (150mg twice daily), eradication of *H. pylori* infection was significantly higher in the former case (100% vs 85.7%, $p<0.005$) [36]. However, a 2002 study reported no improvements in the cure rates of *H. pylori* following a 7-day triple regimen after introduction of zinc L-carnosine [37]. More recently, a 2017 randomized clinical trial compared a 14-day triple regimen (twice daily amoxicillin 1g + clarithromycin 500mg + omeprazole 20mg) with or without zinc L-carnosine 150mg twice daily; the authors found a significantly higher eradication rate in the former group in the per-protocol analysis (83.3% vs 61.4%, $p<0.01$), without significant side effects. In fact, a 75mg twice daily addition of zinc L-carnosine to the triple therapy was sufficient to produce significantly higher eradication rates [38]. In an open label trial, a modified quadruple therapy (zinc L-carnosine 75 mg + bismuth citrate 240 mg + amoxicillin 1g + clarithromycin 500mg + esomeprazole 40mg twice daily for 10 days) was significantly more effective, compared to a 14-day triple regimen alone (eradication rate 93.5% vs 69.6%, $p=0.003$) [39]. Furthermore, a non-inferiority trial, comparing two quadruple therapy regimens, one containing zinc L-carnosine (amoxicillin 1g + clarithromycin 500mg + esomeprazole 20mg + zinc L-carnosine 75mg twice daily) and one containing bismuth (amoxicillin 1g + clarithromycin 500mg + bismuth potassium citrate 220mg + esomeprazole 20mg twice daily), is currently underway in China; the primary outcome is the rate of H. *pylori* eradication [40]. If this and other studies should confirm an advantage to the addition of zinc-L-carnosine to standard anti-*H. pylori* treatment, the use of this compound could be recommended, in light of

the declining eradication rates and increasing H. *pylori* resistance to antibiotics worldwide [41].

Chemoradiotherapy-induced oral mucositis

A number of studies have assessed the beneficial effects of zinc L-carnosine on the incidence and severity of oral mucositis, as well as prevalence of symptoms and use of analgesics in cancer patients receiving chemoradiotherapy. Negative effects on adverse event rate or primary treatment outcomes have not been reported [42, 43, 44].

A recent study compared a prospective cohort of head and neck cancer patients who received oral rinses with zinc L-carnosine during radiotherapy course with a retrospective cohort treated with sodium gualenate hydrate. The authors reported a decreased incidence of grade 3 oral mucositis (29% vs. 40% respectively, p=0.42) and of related symptoms (39.3% vs. 60.7% respectively, p=0.18), although both not statistically significant [45]. In a similar study, confronting oral rinse with zinc L-carnosine and azulene, incidence was significantly decreased (16.5% vs. 52%, p<0.001). The duration of radiotherapy was significantly shorter in the treated group compared to controls (51.5 days vs 56.0 days; HR 5 0.557; 95%CI=0.357-0.871; p=0.0149), with a shorter median time to discharge after completion of radiotherapy (5 days vs 10 days; HR 5 0.604; 95%CI=0.386–0.946; p=0.0276) [46]. Differences in overall survival rates were not statistically significant in both studies. In a another recent prospective study, incidence and grade of oral mucositis (25.8 vs. 94.8% and 0.6 vs. 1.4 respectively, p<0.01), as well as prevalence of the use of non-opioid analgesics (16.1% vs. 89.5%, p<0.01) in patients receiving high-dose chemotherapy for hematopoietic stem cell transplant, were significantly reduced after premedication with zinc L-carnosine suspension, compared to controls. No difference in adverse event incidence was reported; however, controls did not receive a placebo [47]. Similar results were observed in similar settings in pediatric patients, showing significantly reduced post-chemotherapy grade ≥3 oral mucositis incidence: (20% vs. 83.3, p=0.035) [48]. Even more recently, a

multi-institutional randomized controlled study on 91 patients with hematological malignancy receiving chemotherapy assessed the effectiveness of zinc L-carnosine in the prevention of oral mucositis. Patients were assigned to preventive treatment (before and during chemotherapy) or therapeutic treatment upon development of grade 2 stomatitis. Prevention showed significantly reduced incidence of grade ≥2 mucositis at 5 weeks (22% vs 44.7%, *p*=0.025); however incidence of severe mucositis (grade ≥3) was not significantly affected [49].

Thus, zinc-L-carnosine seems to offer a great advantage in terms of prevention of oral mucositis in patients undergoing chemoradiotherapy at the cost of little to no adverse events.

Chemoradiotherapy-induced esophagitis/enteritis

A recent study evaluated the efficacy of zinc L-carnosine in the prevention of chemoradiation-induced esophagitis in patients with non-small cell lung cancer receiving oral alginate. It found that the development of grade ≥2 radiation esophagitis was significantly retarded in treated subjects (HR 0.397; 95%CI, 0.160-0.990; *p*=0.047). The incidence of grade ≥2 radiation esophagitis at the time point of 40 Gy irradiation was significantly reduced (26.3% vs. 63.2%, *p*=0.05). No effect on tumor response of adverse events was observed [50].

In mice, treatment with oral zinc L-carnosine before or after radiation exposure has shown protective effects in the intestinal mucosa, compared with no administration; jejunal damage, expressed as an apoptotic index, was drastically reduced (0.044 vs. 0.106 respectively, *p*=0.01). This effect was shown to be present even at the basal portions of the crypt, which are critical for epithelial regeneration [51]. Similarly, in another murine model of 5-fluorouracil induced enteritis, the authors reported restored villous integrity and crypt proliferation index after oral zinc-L-carnosine administration, in a dose-dependent manner [52]. However, no human studies exist on the possible role of zinc-L-carnosine in preventing or reducing radiotherapy-induced intestinal injury.

An Italian single-center double-blind, randomized, placebo-controlled human trial on the efficacy of zinc L-carnosine in maintaining remission of symptomatic gastro-esophageal reflux disease (GERD) after an 8-week proton pump inhibitor (PPI) course is ongoing but no results have yet been published [clinicaltrials.gov, NCT 03467438, url: https://clinicaltrials.gov/ct2/show/NCT03467438].

NSAID enteritis

After an initial *in vivo* demonstration of the pharmacological effects of zinc L-carnosine on the small intestinal epithelium [14], a randomized study evaluated the effects of oral zinc L-carnosine in patients assuming long-term low-dose aspirin, by means of small bowel capsule endoscopy [53]. In total, 20 patients with aspirin-induced mucosal injury were randomized to zinc L-carnosine 150mg daily or no treatment (controls). At follow up capsule endoscopy at 4 weeks, treated patients showed significantly lower numbers of erosion/ulcers compared to baseline, while controls did not show significant differences.

It should be noted that small intestinal enteropathy is a frequent adverse event during the course of NSAID treatment, being observed in approximately 10-80% of subjects [54]. Thus, these observations seem of particular relevance since, whereas in the upper gastrointestinal tract antisecretory or cytoprotective agents may be used to reduce the occurrence of mucosal damage, no other agents beyond zinc-L-carnosine have currently the potential to prevent small intestinal mucosal injury during NSAIDS therapy.

Effects on intestinal mucosal permeability

Beneficial effects on mucosal permeability have been described both i*n vitro* and *in vivo* [55, 56], mainly through an improvement of epithelial resistance and tight junction structure. In a double-blind placebo-controlled study in healthy volunteers, increase in intestinal permeability after heavy exercise was moderated after 14-day zinc L-carnosine

treatment. Treated patients showed a 71% reduction compared to controls, as measured by the lactulose/rhamnose ratios [56]. Similarly, indomethacin induced a 3-fold increase in intestinal permeability in human controls but not in subjects taking zinc L-carnosine [55].

These observations need to be confirmed in the clinical setting, due to the relevance of mucosal permeability impairment in the pathophysiology of organic and functional bowel disorders.

Colitis

Zinc L-carnosine has been successful in controlling inflammation in experimental animal models of colitis [57], with efficacy comparable to sulfasalazine in one report [4]. Several mechanisms of action have been suggested, such as antioxidant activity [4] and heat shock protein induction [19]. More recently, an immunomodulatory calcineurin-mediated effect of zinc L-carnosine has been shown to reduce colonic inflammation in experimental murine colitis. In this study, inhibition of calcineurin, a key enzyme in T-cell activation and cytokine expression, such as IL-2, IFN-γ, and TNF-α, as well as a macroscopic reduction of inflammation severity was observed after rectal administration [20].

Rectally administered zinc L-carnosine has shown efficacy against acute radiation-induced proctitis in rats: endoscopic and histopathologic findings were significantly less severe after a 10-day post-irradiation treatment [58]. In a dextran sodium sulfate-induced ulcerative colitis (UC) mouse model, zinc L-carnosine significantly reduced diarrhea and release of inflammatory factors, such as TNF-α and NF-κB [59].

In a recent randomized, placebo-controlled, investigator-blinded trial, zinc L-carnosine enemas (150mg once daily) were administered to patients affected by ulcerative colitis of the distal colon and compared to affected controls receiving placebo; both groups received standard induction therapies. In the treated group, Matt's endoscopic scores at 1 week were significantly improved in the rectum, sigmoid and descending colon; among controls scores improved in the sigmoid only. Full Mayo

scores decreased significantly in both groups (treated group, 9.1±1.6 vs. 5.8±2.7, $p<0.001$; controls 8.9±1.7 vs 7.4±2.1, $p=0.009$); however, in the treated group, clinical response was significantly better than in controls [60].

These observations raise the possibility of zinc-L-carnosine application in both acute and chronic colitis.

Hemorrhoidal disease

A multicenter uncontrolled open clinical trial has recently assessed the efficacy of zinc L-carnosine in alleviating symptoms in hemorrhoidal disease. Treated patients showed a marked reduction in bleeding rates (71.4% at baseline, 9.5% at week 4, $p=0.005$), pain VAS scores and overall severity of symptoms using a composite score (Hemorrhoidal Disease Symptom Score) already at 2 weeks, maintaining response at the 4 week assessment [61].

Endoscopic submucosal dissection

Endoscopic mucosal dissection (ESD) is a relatively widespread technique for the removal of gastrointestinal neoplasia, allowing higher resection rates of more advanced and/or larger lesions than classic endoscopic mucosal resection. Owing to its more invasive nature, ESD causes deeper and larger iatrogenic ulcers, which pose a higher risk for delayed complications, particularly in the stomach. Proton pump inhibitor treatment is the mainstay of prevention in this context. However, other mucoprotective agents, like rebamipide, have been proposed.

The effect of zinc L-carnosine (150mg daily) in addition to a standard PPI regimen (lansoprazole 30mg daily) on ESD-induced gastric ulcer healing was evaluated in a 2010 prospective study on 163 patients. Blinded endoscopic assessment at 8 weeks showed significantly better ulcer healing, with protrusion of the ulcer base in only 1.3% of cases in the

combined treatment group, compared to PPI alone (20.7%, p<0.001) [62]. In a similar study, combined treatment also resulted in higher ulcer healing rates compared to single dose omeprazole (95.7% vs. 88.9%) [63]. A recent randomized trial on 218 patients, published in 2021, aimed to compare the efficacy of PPI + zinc L-carnosine to a PPI + rebamipide regimen in the healing of ESD-induced gastric ulcers. The authors found similar healing rates at 4 weeks in both treatment groups (90.3% vs 91.4% respectively) [64].

Acceleration of mucosal healing after endoscopic procedures by locally instilled and orally administered zinc-L-carnosine may reduce the rate of complications (e.g bleeding, stenosis) and post-procedure symptoms, significantly improving outcomes and patient quality of life.

Safety

Zinc homeostasis aims to maintain constant intracellular levels, with a relatively narrow range of plasma concentration (0.7-1.6 mg/L), owing to the adaptive interplay of intestinal absorption, excretion in the feces and urine, as well as intracellular retention. An important regulation mechanism is the metallothionein chelation of zinc within the enterocyte. Following an increase of dietary intake, excess zinc is retained in the enterocytes, not reaching the portal circulation, and is excreted back into the gut with physiological apoptosis. A similar process in the liver prevents excess zinc from reaching the systemic circulation, excreting it in the bile. These processes are adaptive, permitting homeostasis within a wide range of daily intakes (1.4-19.6 mg/day). In the US, recommended dietary allowance is 8-11 mg/day, with a tolerable upper limit of 40 mg daily in adults [2]. Acute ingestion of very high doses of zinc (>200mg) can produce emesis and nausea [52]. However, high luminal concentrations of zinc are known to impair copper absorption in the gut, rendering copper supplementation necessary in some cases [2].

Zinc L-carnosine is a 1:1 chelate compound of zinc and L-carnosine, approximately 23% and 77% by weight respectively. Typical therapeutic

regimens of zinc L-carnosine in humans range from 75mg to 300mg daily, usually employed over limited periods of time [31, 38, 56]. This would represent a daily intake of approximately 17-69 mg of zinc. Although zinc absorption in the gut is dependent on several factors, including luminal concentrations, it can be saturated, particularly at higher doses [26, 27]. Animal studies have shown that approximately 85% of zinc released from its L-carnosine complex persisted in the feces, while only 0.3% was excreted in urine, with an absorption rate of approximately 11% at 50 mg/kg [8, 26]. Long term toxicity tests in animals reported side-effects at very high doses (>300 mg/kg for 13 weeks), including salivation, decrease in hemoglobin and reticulocyte count, elevated liver enzymes and diarrhea. Interactions with some drugs (such as penicillamine and levothyroxin) have also been reported [65, 66].

In human studies, zinc L-carnosine at standard therapeutic doses (up to 300 mg daily) did not show significant adverse effects [31, 36, 37, 47, 49, 60]. However, some cases of transient laboratory abnormalities, mainly increased liver enzymes have been noted [31, 38]. Most cases of adverse events (e.g. dizziness) were reported in combination with a triple or bismuth-containing regimen for H. *pylori* eradication, mostly at higher doses of zinc L-carnosine [36, 37, 38].

CONCLUSION

The data available so far seem to indicate that zinc L-carnosine is more than the sum of its parts: although zinc is the major active component regarding mucosal protection and healing properties, some evidence exist to suggest an additional carnosine-dependant cytoprotective effect. In addition, the L-carnosine complex is shown to be a far more capable vehicle for tissue delivery than zinc salts, such as zinc sulfate, because of its adhesive and targeting properties, particularly on damaged tissue, that result in better permanence and selectivity for mucosal injury.

Animal and human studies show that zinc L-carnosine is useful to both prevent and treat mucosal injury through local anti-inflammatory, anti-oxidant and cytoprotective action. These properties, being independent from classical acid-suppressing or prostaglandin-mediated pathways, should not interfere with standing pharmacological treatments or limit the compound's usefulness to gastroprotection, but are in principle applicable to all mucosal tissue injury. This potentially makes zinc L-carnosine a "universal" drug for gastrointestinal mucosal protection, both alone and in combination with other treatments, all the more because of its reported safety. Furthermore, recent studies suggest a potential beneficial role in controlling non-inflammatory stress-induced mucosal responses, which may underpin conditions or syndromes classically classified under the functional disease spectrum. Potential applications may range from more "classical" indications, such as radiation/chemotherapy-induced mucositis and peptic ulcers, to more novel ones, like the prevention of ESD complications or ulcerative proctitis.

Indeed, some of the aforementioned applications are of particular interest to gastroenterologists. Aside from its principal application in the treatment of peptic ulcers, several promising uses have been identified. An obvious extension to the standard indications of zinc L-carnosine is its general utility in promoting ulcer healing outside the classical peptic ulcer setting. Emerging uses in humans in the treatment ESD-induced gastric ulcers have shown promising results in accelerating healing rates, potentially reducing procedure-related complications and improving tolerability and outcomes. In this specific context, uses could extend to other anatomical sites, such the esophagus or the rectum, where delivery of the compound is currently practical.

The general efficacy of zinc L-carnosine in preventing pharmacological and chemo/radiotherapy-induced mucosal damage further extends the potential applications of the compound. Use of zinc L-carnosine is increasing in the context of gastro-esophageal reflux disease, although in the absence of direct evidence of efficacy in classical GERD. In this setting, its ulcer-healing and mucoprotective properties may be of

obvious utility in the treatment of erosive esophagitis, based on available data. Furthermore, its reported positive effects on mucosal permeability may be of benefit in the symptomatic first-line and chronic treatment of symptomatic GERD, irrespective of erosive disease, including esophageal hypersensitivity.

Impairment of the gut barrier and excessive mucosal permeability are pathophysiologically relevant conditions in functional intestinal disorders, such as Irritable Bowel Syndrome (IBS). Although several possible factors can contribute to impaired barrier function, the latter seems to mediate visceral hypersensitivity, gut-brain interaction and development of symptoms. Zinc L-carnosine has been shown to improve tight junction structure and gut barrier function, and thus may have a role in the management of IBS and IBS-like disorders.

Major factors limiting the potential use of zinc L-carnosine in the gastroenterological setting are primarily liked to technical difficulties in delivery and dose. While generally safe and well-tolerated, zinc L-carnosine is a topical agent, locally releasing its constituents once bound to damaged mucosa. Carnosine is rapidly metabolized locally, while zinc could potentially affect zincemia when taken in higher than therapeutic doses. Thus, oral delivery to the small intestine and colon would be impractical with current formulations. However, optimized enteric coated, pH/time-dependent formulations could potentially overcome some of these obstacles.

Both liquid and capsule formulations of zinc L-carnosine are commercially available. In Italy, existing formulations contain 37.5mg per capsule/sachet with the addition of sodium alginate and magnesium hydroxide. It is possible that alginate further promotes adhesiveness, especially in sites where contact times are presumably short, such as the esophagus. Antacids could also promote zinc L-carnosine efficacy, temporarily suppressing luminal acidity and thus preventing dissociation of the compound before it reaches the damaged epithelium, limiting its dispersion and prolonging local activity.

To date, available data particularly in humans, are limited or dated. Despite the soundness of the theoretical and experimental framework regarding the usefulness and efficacy of zinc L-carnosine for the prevention and treatment of mucosal disease, additional studies on humans are needed to further strengthen the observations on the efficacy and long-term safety of the compound. Emerging data on novel potential indications are particularly intriguing and warrant for further targeted clinical research.

AUTHOR CONTRIBUTIONS

KE: conception, bibliographic research, drafting of the paper, critical revision, final approval of the submitted version

MN: conception, critical revision, final approval of the submitted version

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Summary of available human studies of zinc L-carnosine applications in GI disease.

Author	Aim	Design	Participants (n)	Duration	Dose	Main outcomes
Miyoshi A, et al. 1992a [28]	Effect on gastric ulcers	Multicenter double-blind controlled trial	299	8 weeks	ZnC 75 mg BID (n=148) vs. cetraxate 400 mg BID (n=151)	Normal endoscopy at 8 weeks: 60.4% (ZnC) vs. 46.2% (cetraxate, $p<0.05$); markedly improved symptoms at 8 weeks: 50.4% (ZnC) vs. 37.0% (cetraxate, $p<0.05$).
Miyoshi A, et al. 1992b [29]	Effect on gastric ulcers	Multicenter double-blind dose-finding study	229	8 weeks	50 mg BID, 75 mg BID or 100 mg BID	Marked improvement in 75.4% (50 mg BID), 71.6% (75 mg BID) and 78.5% (100 mg BID)
Morise K, et al.	Effect on gastric ulcers	Open label clinical	64	8 we	75 mg BID	Normal endoscopy at 8 weeks: 67.4%; significantly improved symptoms at 8 weeks: 69.8%

1992 [30]		trial		eks		
Hayakawa A, et al. 1992 [31]	Effect on gastric ulcers	Double-blind clinical trial	44	8 weeks	75 mg BID	Normal/nearly normal endoscopy at 8 weeks: 80.0%; significantly improved symptoms at 8 weeks: 60.7%
Nakajima M. 1997 [32]	Effect on gastritis	Multicenter double-blind study vs. sucralfate	348	2 weeks	ZnC 50 mg TID vs. sucralfate 900 mg TID	Endoscopic improvement: 75.5% (ZnC) vs. 71.3% (sucralfate); symptomatic improvement: 81.2% (ZnC) vs. 78.4% (sucralfate)
Kashimura H, et al. 1999 [36]	Efficacy in H. *pylori* eradication treatment	Open label controlled clinical trial	66	1 week	ZnC 150 mg BID + triple therapy (n=35) vs. triple therapy alone (n=31)	H. *pylori* eradication rates (per protocol): 100% (ZnC + triple therapy) vs. 86% (triple therapy alone, $p<0.05$)
Isomoto H, et al.	Efficacy in H. *pylori* eradication	Open label controlled	156	1 we	ZnC 150 mg BID + triple therapy (n=53) vs. sofalcone 100 mg TID + triple therapy	H. *pylori* eradication rates (per protocol): 84.9% (ZnC + triple therapy) vs. 94% (sofalcone + triple therapy) vs. 81.1% (triple therapy

2005 [37]	treatment	clinical trial		ek	(n=49) vs. triple therapy alone (n=53)	alone, *p*not significant regarding addition of ZnC)
Tan B, et al. 2017 [38]	Efficacy in H. *pylori* eradication treatment	Open label controlled clinical trial	332	2 weeks	ZnC 75 mg BID + triple therapy (n=113) vs. ZnC 150 mg BID + triple therapy (n=108) vs. triple therapy alone (n=111)	H. *pylori* eradication rates (per protocol): 81.1% (ZnC 75 mg BID + triple therapy) vs. 83.3% (ZnC 150 mg BID + triple therapy) vs. 61.4% (triple therapy alone, *p*<0.01)
Wu D, et al. 2020 [40]	Efficacy in H. *pylori* eradication treatment	Single-center, single-blind, non-inferiority trial	158	2 weeks	ZnC 75 mg BID + triple therapy (n=79) vs. bismuth citrate 220 mg BID + triple therapy (n=79)	H. pylori eradication rates *(ongoing)*
Ibrahim N, et al. 2022 [39]	Efficacy in H. *pylori* eradication treatment	Open label controlled clinical trial	92	-	10-day ZnC 75 mg BID + bismuth citrate 240 mg BID + triple therapy (n=46) vs. 14-day triple therapy alone (n=46)	H. pylori eradication rates (per protocol): 93.5% (ZnC 75 mg BID + bismuth citrate 240mg + triple therapy) vs. 69.6% (triple therapy alone, *p*=0.003)
clinicaltrials.gov, NCT	GERD remission maintenanc e	Single-center, double-blind,	80	12 we	ZnC 75 mg BID vs. placebo	GERD remission rates at 4, 8 and 12 weeks *(ongoing)*

03467438		placebo-controlled trial		eks		
Masayuki F, et al. 2002 [42]	Prevention of radiotherapy-induced oral mucositis	Open label study	19	-	ZnC oral suspension (swallowed) (n=11) vs. no treatment (n=8)	Post-radiotherapy grade 3 stomatitis incidence: 9% (ZnC) vs. 50% (no treatment, *p*=0.046)
Watanabe T, el al. 2010 [43]	Prevention of radiochemotherapy-induced oral mucositis	Randomized controlled trial	31	-	ZnC 18.75 mg rinse QID (swallowed) (n=16) vs. azulene rinse QID (n=15)	Post-chemoradiotherapy grade 3 stomatitis incidence: 6.3% (ZnC) vs. 66.7% (azulene, *p*<0.001)
Hayashi H, et al. 2014 [44]	Prevention of chemoradiotherapy-induced oral mucositis	Retrospective study	47	5 weeks	ZnC 18.75 mg rinse QID (swallowed) (n=36) vs. azulene rinse QID (n=11)	Post-chemoradiotherapy grade 3 stomatitis incidence: 0% (ZnC) vs. 45% (azulene, *p*<0.01)
Doi H, et al. 2015	Prevention of radiotherap	Retrospective case-matched	61	-	ZnC 37.5 mg rinse QID (not swallowed) + sodium gualenate hydrate	Post-radiotherapy grade 3 stomatitis incidence: 29% (ZnC + sodium gualenate hydrate) vs. 40% (sodium gualenate hydrate,

[45]	y-induced oral mucositis	study			mouthwash (n=31) vs. sodium gualenate hydrate mouthwash (n=30)	not significant, *p*=0.42)
Suzuki A, et al. 2016 [46]	Prevention of radiotherapy-induced oral mucositis	Retrospective study	104	-	ZnC 18.75 mg rinse QID (swallowed) (n=79) vs. azulene rinse QID (n=25)	Post-radiotherapy grade 3 stomatitis incidence: 16.5% (ZnC) vs. 52% (azulene, *p*<0.001)
Hayashi H, et al. 2016 [47]	Prevention of chemotherapy-induced oral mucositis	Retrospective study	66	-	ZnC 18.75 mg rinse QID (swallowed) (n=31) vs. ZnC 18.75 mg lozenge QID (n=16) vs. no treatment (n=19)	Post-chemotherapy grade ≥2 stomatitis incidence: 22.6% (ZnC rinse) vs. 12.5% (ZnC losenge) vs. 73.7% (no treatment, *p*<0.01)
Funato M, et al. 2018 [48]	Prevention of chemotherapy-induced oral mucositis	Retrospective study in children	16	-	ZnC 18.75 mg rinse QID (swallowed) (n=10) vs. azulene rinse QID (n=6)	Post-chemotherapy grade ≥3 stomatitis incidence: 20% (ZnC) vs. 83.3% (azulene, *p*=0.035)
Kitagawa J, et al.	Prevention of chemothera	Multicenter randomiz	88	5 we	Preventive ZnC 18.75 mg lozenge QID (n=41) vs. therapeutic ZnC 18.75 mg	Post-chemotherapy grade ≥2 stomatitis incidence: 22% (preventive ZnC) vs. 44.7% (therapeutic ZnC, *p*=0.025)

2021 [49]	py-induced oral mucositis	ed controlled trial		eks	lozenge QID for established moderate mucositis (n=47)	
Yanase K, et al. 2015 [50]	Prevention of chemoradiotherapy-induced esophagitis	Retrospective study	38	-	ZnC 50 mg + sodium alginate TID (n=19) vs. sodium alginate TID (n=19); in both groups aluminum-magnesium hydroxide gel TID	Post-chemoradiotherapy grade ≥2 esophagitis incidence: 26.3% (ZnC + alginate) vs. 63.2% (alginate) at the 40 Gy irradiation time point (p=0.05); 42.1% (ZnC + alginate) vs. 63.2% (alginate) at the 60 Gy time point (not significant, p=0.33)
Watari I, et al. 2013 [53]	Effect on low-dose aspirin-induced small-bowel mucosal injury	Randomized controlled study	20	4 weeks	Znc 150 mg QD (n=10) vs. no treatment (n=10)	At follow-up capsule endoscopy: significant decrease in the number of erosions/ulcers and total mucosal injuries from baseline in the ZnC group (p<0.01) but not in controls
Mahmood A, et al. 2007 [55]	Effect on gut permeability	Double-blind placebo-controlled crossover study	10	1 week	ZnC 37.5 mg BID vs. placebo; in both groups indomethacin 50 mg TID in the final 5 days	Lactulose/rhamnose ratios in urine in response to indomethacin: no increase from baseline (ZnC) vs. threefold increase from baseline (placebo, p<0.01)
Davison G,	Effect on gut	Double-blind	8	2 w	ZnC 37.5mg + placebo BID vs. colostrum 10gr + placebo	Lactulose/rhamnose ratios in urine in response to exercise at 2 weeks: 1.8-fold increase from

et al. 2016 [56]	permeability	placebo-controlled crossover study		eeks	BID vs. ZnC 37.5 mg + colostrum 10 gr BID vs. placebo alone BID; exercise at day 2 and 14	baseline (ZnC or colostrum) vs. 1.4-fold (ZnC + colostrum) vs. 3.1-fold increase from baseline (placebo, p<0.01)
Itagaki M, et al. 2014 [60]	Efficacy in active ulcerative colitis	Single-blind placebo-controlled randomized trial	28	1 week	ZnC 150 mg enema QD (n=18) vs. placebo (n=10); patients in both groups received 5-ASA/steroid/immunomodulator/antiTNF treatment	Significant improvement of endoscopic scores from baseline in the ZnC group (p<0.05) but not in placebo; clincal response 70.6% (ZnC) vs. 10% (placebo, p=0.002)
Pietroletti R, et al. 2022 [61]	Efficacy in hemorrhoidal disease	Multicenter open label clinical trial	21	4 weeks	ZnC rectal ointment TID	Significant reduction at 4 weeks in bleeding rates (9.5% vs. 71.4%, p=0.005), pain VAS scores (p=0.018) and Hemorrhoidal Disease Symptom Scores (p<0.001) from baseline
Inaba T, et al. 2010 [62]	Effect on post-ESD ulcers	Randomized controlled trial	154	8 weeks	ZnC 150 mg + lansoprazole 30 mg QD (n=77) vs. lansoprazole 30 mg QD (n=77)	Significant improvement of ulcer healing scores in the ZnC group (p<0.001); ulcer base protrusion identified in 1.3% (ZnC + PPI) vs. 20.7% (PPI, p<0.001)
Yoshida N,	Effect on post-ESD	Controlled trial	50	8 w	ZnC 150 mg + omeprazole 20 mg QD (n=23) vs.	Ulcer healing rates: 95.7% (ZnC + PPI) vs. 88.9% (PPI)

et al. 2013 [63]	ulcers			eeks	omeprazole 20 mg QD (n=27)	
Jung DH, et al. 2021 [64]	Effect on post-ESD ulcers	Randomized controlled trial	210	4 weeks	ZnC 75 mg BID + pantoprazole 40 mg QD (n=104) vs. rebamipide 100 mg TID + pantoprazole 40 mg QD (n=106)	Not significant differences between groups: 90.3% (ZnC + PPI) vs. 91.4% (rebamipide + PPI)
ZnC: zinc L-carnosine						
ESD: Endoscopic submucosal dissection						