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AN ENDOGENOUS DEFENSIVE CONCEPT, RENEWED CYTOPROTECTION/ADAPTIVE CYTOPROTECTION: INTRA(PER)-ORAL/INTRAGASTRIC ADMINISTRATION OF STRONG ALCOHOL IN RAT. INVOLVEMENT OF PENTADECAPEPTIDE BPC 157 AND NITRIC OXIDE SYSTEM

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With intra(per)-oral strong alcohol application at the tongue, swallowed, we renewed Robert's stomach cytoprotection/adaptive cytoprotection concept. We assessed strong (96%) alcohol-induced severe or minute lesions in stomach, tongue-esophagus-stomach-duodenum lesions, and sphincter pressure (lower esophageal and pyloric) upon administration intragastrically (at 1 h) or intra(per)-orally at the tongue, and swallowed (at 1, 5, 15, 30 min; and 1, 2, 24 h). The assessment also included combined administrations (intra(per)-oral at the tongue, swallowed and intragastric (at 1 h)). Immediate post-alcohol intraperitoneal medication (mg/kg) was the stable gastric pentadecapeptide BPC 157 (0.01, 0.00001; a Robert's cytoprotection mediator; with a therapeutic effect), NOS-blocker L-NAME (5), and NOS-substrate L-arginine (100 mg), (NO-system involvement). After intragastric strong alcohol administration, severe stomach ulcerations appeared along with widespread tongue, esophagus, duodenum redness, and minimal sphincter pressures. By contrast, a particular syndrome (immediate overlapping of cytoprotection/adaptive cytoprotection) (minute gastric lesion or largely attenuated hemorrhagic ulceration, tongue affected, minute esophageal and duodenal lesions, but with intact mucosa; sphincters pressures lowered) appeared after intra(per)-oral administration (1 min-24 h) as well as after combined administrations (intra(per)-oral + intragastric). BPC 157 apparently cured all alcohol-lesions, amplified the spontaneously initiated strong mucosal beneficial effect, rescued sphincter pressures; NO-agents (L-arginine (slight mucosal amelioration) and L-NAME (aggravation)) showed NO-system involvement, but no comparable effects on dropped sphincters pressures. In conclusion, minute gastric lesions (with oral application of strong alcohol at the tongue and swallowed, without, or with intragastric application of strong alcohol) renew and revise Robert's stomach cytoprotection/adaptive cytoprotection concept. The tongue becomes a new initial target, resulting in spontaneous reversal of strong alcohol-stomach lesions. BPC 157 therapy functions also within the redirected complexity of Robert's stomach cytoprotection/adaptive cytoprotection concept.

Key words: *cytoprotection, pentadecapeptide BPC 157, alcohol, oral application, esophageal injury, stomach lesions, L-arginine, L-N^G-nitroarginine methyl ester, adaptive cytoprotection*

INTRODUCTION

With strong oral alcohol application at the tongue, swallowed, without or with strong alcohol intragastric application, the present study would redirect the complexity of Robert's stomach cytoprotection/adaptive cytoprotection (1-3).

This would define the tongue as a new initial target. And thereby, the potential of the renewed concept can be recorded through a spontaneous reversal of strong alcohol-stomach lesions and then, through the effects of BPC 157 (4-16), and NOS-agents NOS-blocker L-NAME, NOS-substrate L-arginine (NO-system involvement) (10).

Long held as one of the most advantageous concepts and general acceptance, Robert's cytoprotection stomach entails the intragastric application of necrotizing agents through a tube inserted into the rat stomach, as a direct assault to the stomach (1-3). Prophylaxis as a therapy has defined antiulcer agents as a class of cytoprotective agents; however, their effectiveness only when given before alcohol remains a shared limitation (1-3, 17). Furthermore, an essential physiologic importance stems from defining stomach cytoprotection (severe stomach lesion; intragastric *strong* alcohol → *severe* stomach lesions) and adaptive cytoprotection concept (*severe* stomach lesion reversed to *mild* stomach lesion; the original concept 'intragastric *strong* alcohol → *severe* stomach lesions' reversed by particular pretreatment (intragastric *mild* alcohol → *mild* stomach lesions) to 'intragastric strong alcohol → mild stomach lesions')) (1-3). Conceptually, a time pitfall exists between cytoprotection and adaptive cytoprotective responses, and thereby a short critical, defenseless period in the stomach without protection ability (1-3). After the initial response to the initial direct injury (cytoprotection), the afforded response against the repeated direct injuries (adaptive cytoprotection) does not start immediately, and thereby in meantime, special threat to the stomach temporarily left without

any defense (1-3). In addition, the concepts permit extension. Robert's direct cytotoxic killing of gastric cells by direct insult acknowledges cytoprotection in other organs (1-3), *i.e*., liver, and pancreas, as the organoprotection concept (18-20), and a class of cytoprotective agents acting there, as the class of organoprotective agents (*i.e*., prostaglandins, somatostatin, sulfhydryls (18-20) while BPC 157 at the best fits with cytoprotection/organoprotection requirements (4-16)). Also, an extension from stomach epithelium protection to stomach endothelium protection (and thereby concept background stomach endothelium → stomach epithelium protection) (21-24) permits cytoprotective agents as an endothelium protectants class (although this was not originally claimed) (21-24), and consequently, application in thrombosis and bleeding disorders (5, 8, 25-28). A peculiar point was BPC 157 effectiveness to rescue rats with ischemic/reperfusion colitis or occluded inferior caval vein, through rapid activation of collateral circulation (arcade vessels; alternative major veins, *i.e*., left ovarian vein) along with counteraction of free radical formation (25, 29).

However, further theoretical and practical concept consideration in eating/drinking would necessitate the tongue as a new initial target and swallowing to depict a more regular endogenous defensive concept continuously functioning toward the stomach. By contrast, if Robert's concept applies the necrotizing agent (*i.e*., absolute alcohol) intragastrically, delivery goes directly through the inserted orogastric tube into the stomach (procedure that Robert (incorrectly) referred as oral application) (1-3). In reality, it means completely 'unprepared stomach' for such an unusual event. Thus, in our view, while the present concept holds huge and undisputable theoretical and practical impact, the injury course in the stomach itself so far implements only 'unprepared stomach' when direct delivery through a tube inserted into the stomach skips a normal defensive system in the upper part of the gastrointestinal tract (1-3). Thereby, the renewal of the original Robert's concept (1-3) deserves further consideration with 'prepared stomach' with normal defensive system in upper part of gastrointestinal tract properly activated before.

Thus, the renewal of the concept holds 96%-ethanol intra(per)-oral application at the tongue, swallowed, and only the minute stomach lesions. Thereby, a concept that continuously operates in eating/drinking rats along with immediate overlapping cytoprotection/adaptive cytoprotection to maintain continuously mucosal integrity, in unbroken sequence. The stomach lesion presentation, as a clue, would consider the rat tongue (the first target)-esophagus-stomach-duodenum chain of events, and lower esophageal and pyloric sphincter (mal)function.

Finally, if animal precondition occurs, modification of defensive process occurs before its initiation much like in the classic cytoprotection studies (note, standard cytoprotective agents would act only when given before alcohol (1-3)). Rather, the novel concepts distinguishes itself by having an application of the agents' immediately after alcohol application. Specifically, post-alcohol medication would respect ongoing spontaneous defensive course, to affect spontaneous course of the defensive processes once rapidly initiated immediately after alcohol, either to afford or to abrogate. We applied pentadecapeptide BPC 157, known to be novel mediator of Robert's cytoprotection and prototype of novel class of cytoprotective agents acting both before and after alcohol administration (4-16). The administration of NOS-blocker L-NAME and NOS-substrate L-arginine intents to control the process. Agents were given alone and/or together.

MATERIALS AND METHODS

Animals

Male Albino Wistar rats, 200 g b.w., randomly assigned to groups, were used for experiments, approved by the Local Ethics Committee at the School of Medicine (University of Zagreb, Zagreb, Croatia). The ethanol procedure was done after overnight fasting, with water provided until 2 h before the strong alcohol application, or they had food and water *ad libitum* until the end of the experiment (4-16, 30-33). Since no differences were observed, these data were shown together (4-16, 30-33).

Drugs

Pentadecapeptide Gly-Glu-Pro-Pro-Pro-Gly-Lys-Pro-Ala-Asp-Asp-Ala-Gly-Leu-Val, M.W. 1419, named BPC 157 which is a part of the sequence of human gastric juice protein, coded BPC, freely soluble in water at pH 7.0 and in saline, was prepared (Diagen, Slovenia) as described before (4-16, 30-33). The peptide used was 99% high pressure liquid chromatography (HPLC) purity with 1-des-Gly peptide as biologically inactive impurity (4-16,30-33). L-NAME and L-arginine were commercially purchased (Sigma, USA).

Strong alcohol administration procedures

To discern a spontaneous reversal of alcohol-stomach lesions, the procedures were as follows (*Fig. 1*): (i) ethanol 96% was given intraorally, at the tongue, 1.0 ml/rat, and animal was allowed to swallow entire applied volume; (ii) ethanol 96% was given intragastrically in a dose of 1.0 ml/rat, as previously described (4-16, 30-33); (iii) combined administrations. Ethanol 96% was given intra(per)-orally, at the tongue, 1.0 ml/rat, and animal was allowed to swallow all applied volume. Then immediately, ethanol 96% was given intragastrically in a dose of 1.0 ml/rat, as previously described (30-33) and as emphasized before (30-33), excluding possible alcohol dilution as the reason for the lesion attenuation.

Lower esophageal sphincter pressure assessment and pyloric sphincter pressure assessment

As described previously (34-42), manometrical evaluation (cm H_2O) was performed on rats before sacrifice with a water manometer connected to the drainage port of the Foley catheter, as described. The values of 68 – 76 cm H_2O for lower esophageal sphincter and 68 – 74 cm H_2O for pyloric sphincter were considered to be normal as determined before. The proximal side of the esophageal or distal side of the duodenal incision was ligated to prevent regurgitation (34-42).

Medication

Medication (dose/kg b.w.) (pentadecapeptide BPC 157 (10 μg, 10 ng), L-NAME 5 mg, L-arginine 100 mg given alone and/or together, while control received an equivolume of saline (5 ml)) was given intraperitoneally immediately after ethanol application.

The animals were sacrificed at the 1, 5, 15, and 30 min, and 1, 2 and 24 h after intra(per)-oral strong ethanol (i), or at 1 hour after intragastric alcohol instillation (ii) or at 1 hour when after the intra(per)-oral strong alcohol the intragastric instillation of the strong alcohol was immediately given (iii).

Assessment of mucosal injury

Immediately after euthanizing the rats, the tongue, esophagus, stomach, and duodenum were removed and the lesion areas, presenting as the redness, congestion, or frank ulcerations were assessed (mm^2, mean ± SD) (30-33). Representative tissue sections were processed for further histological analysis as described previously (30-33).

Statistical analysis

Statistical analysis was performed by a non-parametric Kruskal-Wallis ANOVA and subsequent Mann-Whitney U-test to compare groups. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Effect of ethanol intra(per)-oral application at the tongue and ethanol swallowed.

The tongue, esophagus, and duodenum presented with extensive redness, while the stomach showed an additional congestion in the whole of the glandular part with preserved mucosa (*Fig. 1A*). The lesions presented from the very beginning and were sustainably maintained until the end of the experiment (*Fig. 2*).

Effect of ethanol intra(per)-oral before ethanol intragastric application alone or combined with strong ethanol administrations.

In comparison with the intragastric administration of the strong alcohol, in rats that initially received intra(per)-oral application at the tongue, swallowed just before the intragastric instillation of the strong alcohol (*Fig. 1A, B*), the stomach showed largely attenuated hemorrhagic ulceration in the whole of the glandular part, while the tongue, esophagus, and duodenum exhibited less extensive redness (*Fig. 3*).

Effect of ethanol applications on fallen sphincters pressures

We noted with the intra(per)-oral application of the strong alcohol, the markedly fallen sphincter pressures with practically preserved stomach mucosa (*Fig. 1*, *Fig. 4, down*). We noted with the intragastric strong alcohol, even more fallen sphincter pressures, and in particular, complete pressure failure in the lower esophageal sphincter (*Fig. 4*, *upper*, *left*), along with largely damaged, ulcerated stomach mucosa. With combined administrations, when the intra(per)-oral strong alcohol was given just before intragastric strong alcohol, the attenuated drop of the sphincter pressures (*Fig. 4*, *upper*, *right*) resembled the attenuated lesions.

Effect of pentadecapeptide BPC 157 applied alone or in combination with L-NAME and L-arginine.

Considering the mucosal presentation, in all tissues, a strong beneficial effect appeared with BPC 157 after intra(per)-oral application of strong alcohol, swallowing, intragastric application of strong alcohol, and after combined administrations intra(per)-oral application of strong alcohol, swallowed plus intragastric

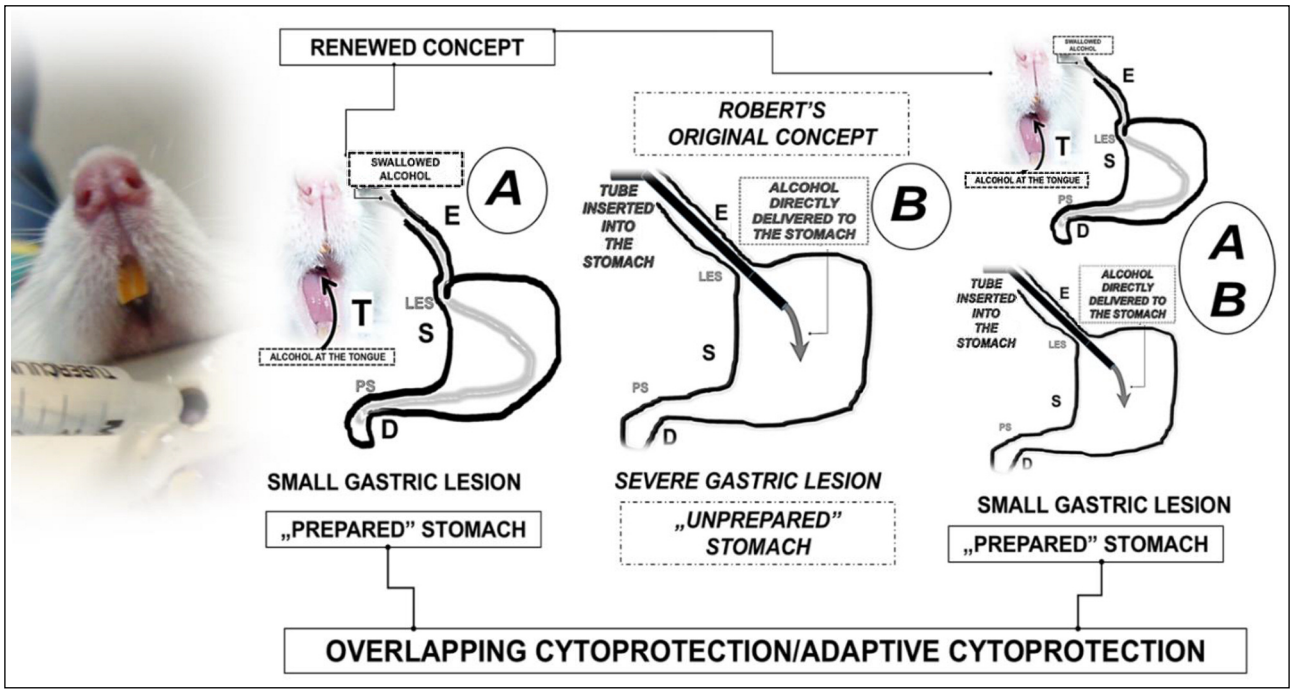

Fig. 1. Renewed concept. Further theoretical and practical concept consideration in eating/drinking would necessitate tongue as a new initial target and swallowing (*left*) to depict a more regular endogenous defensive concept continuously functioning toward the stomach (*A*, *AB*) with per(intra)-oral application of strong alcohol at the tongue, swallowed, without *(A)* or with intragastric application of strong alcohol (*AB*). This would result in a spontaneous reversal of strong alcohol-stomach lesions, and thereby small gastric lesion and a particular syndrome (immediate overlapping of cytoprotection/adaptive cytoprotection). By contrast, if Robert's concept applies necrotizing agent (*i.e*., absolute alcohol) intragastrically, delivery goes directly through the inserted orogastric tube into the stomach (procedure that Robert (incorrectly) referred as oral application) (*B*). In reality, it means completely 'unprepared stomach' to an unusual event, direct delivery through tube inserted into the stomach skips out a normal defensive system in upper part of gastrointestinal tract, and thereby severe gastric lesion (*B*). Thereby, the renewal of the original Robert's concept (*A*, *AB*) deserves further consideration with 'prepared stomach' with normal defensive system in the upper part of gastrointestinal tract properly activated before with per(intra)-oral application of strong alcohol at the tongue, swallowed, without *(A)* or with intragastric application of strong alcohol (*AB*).

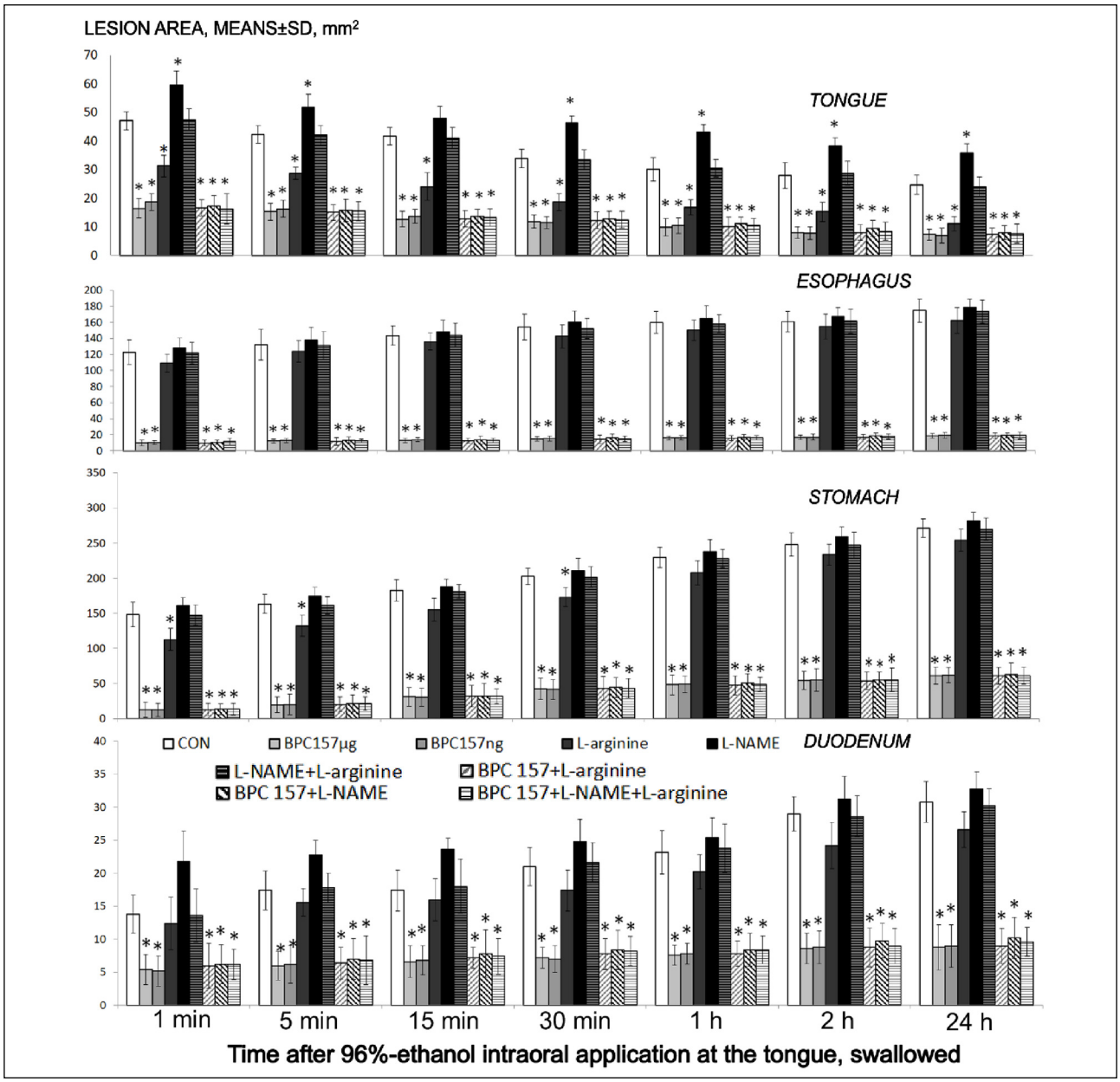

Fig. 2. Lesion area after intra(per)-oral strong alcohol administration. Strong alcohol was given intra(per)-orally, at the tongue, 1 ml/rat, and animal was allowed to swallow all applied volume. Sacrifice was at the 1, 5, 15, 30 min, 1, 2 and 24 hours. Immediate post-alcohol intraperitoneal medication (/kg)included pentadecapeptide BPC 157 (10 μg, 10 ng), L-arginine 100 mg, L-NAME 5 mg given alone and/or together. Control received an equal volume of saline (5 ml/kg) immediately after strong alcohol, and presented minute gastric lesions, tongue affected, minute esophageal and duodenal lesions, as the redness, congestion as described (6, 10-13) but with intact mucosa. BPC 157 apparently cured all alcohol-lesions and amplified spontaneously initiated strong mucosal beneficial effect, and overrode NO-agents effects. NO-agents showed NO-system involvement (L-arginine (therapy effect, continuously affecting tongue, and intermittently affecting stomach, thereby a slight effect) and L-NAME (tongue lesions aggravation) and together, the responses antagonized each other's effects). Frank ulcerations were absent. 10 rats/group at least, mm^2, means ± SD, *$P < 0.05$, at least versus control.

application of strong alcohol. This therapeutic effect was along with an increase of the decreased sphincter values, both lower esophageal and pyloric sphincters, thus a general beneficial effect (*Fig. 2-4*). Given with NO-agents, BPC 157 maintains its therapeutic effect on all mucosa tissue and also apparently rescues sphincters pressures, and thereby, overrides NO-agents effects (*Fig. 2-4*).

Considering the mucosal presentation, it is possible that NO-agents show distinctive NO-system involvement. For illustration, after intra(per)-oral application of strong alcohol, swallowed, L-arginine shows a theraputic effect, continuously affecting tongue, and intermittently affecting stomach, and L-NAME presents tongue lesions aggravation while these responses antagonize each other response. After combined administrations (intra(per)-oral application of strong alcohol, swallowed + intragastric application of strong alcohol) L-arginine resulted in intermittent therapy effect on the esophagus and duodenum and L-NAME led to an apparent aggravation seen with tongue and esophagus lesion. However, these responses antagonized each other's response only in

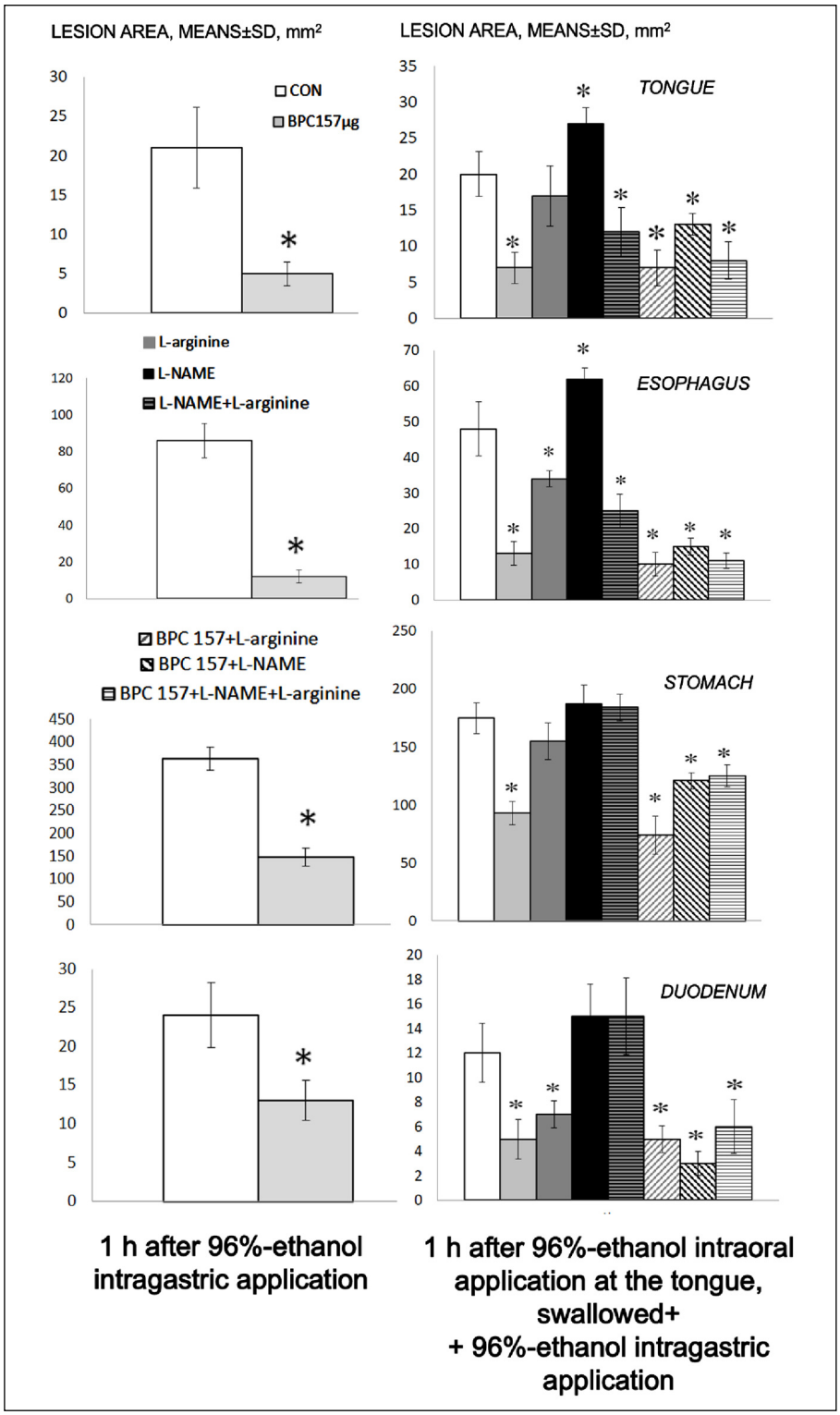

Fig. 3. Lesion area at 1 hour after intragastric strong alcohol administration (1 ml/rat) (*left*) or combined administrations, intra(per)-oral strong alcohol administration (1 ml/rat), swallowed + intragastric strong alcohol administration (1 ml/rat) (*right*). *Left*: Immediate post-alcohol intraperitoneal medication was pentadecapeptide BPC 157 (10 µg). Control received an equal volume of saline (5 ml/kg) immediately after strong alcohol. They presented extensive hemorrhagic ulceration in the whole of the glandular stomach part (as described (6, 10-13)) while the tongue, esophagus, and duodenum exhibited extensive redness. BPC 157 apparently mitigates all alcohol-lesions. *Right*: Immediate post-alcohol intraperitoneal medication includes pentadecapeptide BPC 157 10 µg, L-arginine 100 mg, L-NAME 5 mg, given alone and/or together. Control received an equal volume of saline (5 ml/kg) immediately after strong alcohol, and presented lesions attenuation, largely attenuated hemorrhagic ulceration in the whole of the glandular part. The tongue, esophagus, and duodenum exhibited less extensive redness. BPC 157 apparently decreased all alcohol-lesions, amplified a spontaneously initiated strong mucosal beneficial effect, and overrode the NO-agents effects. NO-agents showed NO-system involvement (L-arginine (intermittent therapy effect on esophagus and duodenum) and L-NAME (an apparent aggravation seen with tongue and esophagus lesion) which antagonized each other's response only in duodenum). 10 rats/group at least, mm², means ± SD, *P < 0.05, at least versus control.

duodenum. These effects were not along with the effect on sphincters pressure. Illustratively, after intra(per)-oral application of strong alcohol, swallowed, NO-agents showed no effect. After combined administrations, NO-agents showed a limited NO-system involvement. Pressure in lower esophageal sphincter (but not in pyloric sphincter), L-arginine further decreased, L-NAME also further decreased while L-NAME + L-arginine rats presented values similar to those observed with controls (*Figs. 2-4*).

Macroscopic and microscopic findings in rats with ethanol intra(per)-oral administration at the tongue and ethanol swallowed.

The major impact was at the tongue (*Figs. 5-7*). After 2 hours, all rats presented mild reactive changes to the surface epithelium, scarce, unevenly distributed accumulations of polymorphonuclear inflammatory cells on the surface of muscle while the controls exhibited more pronounced edema of stroma

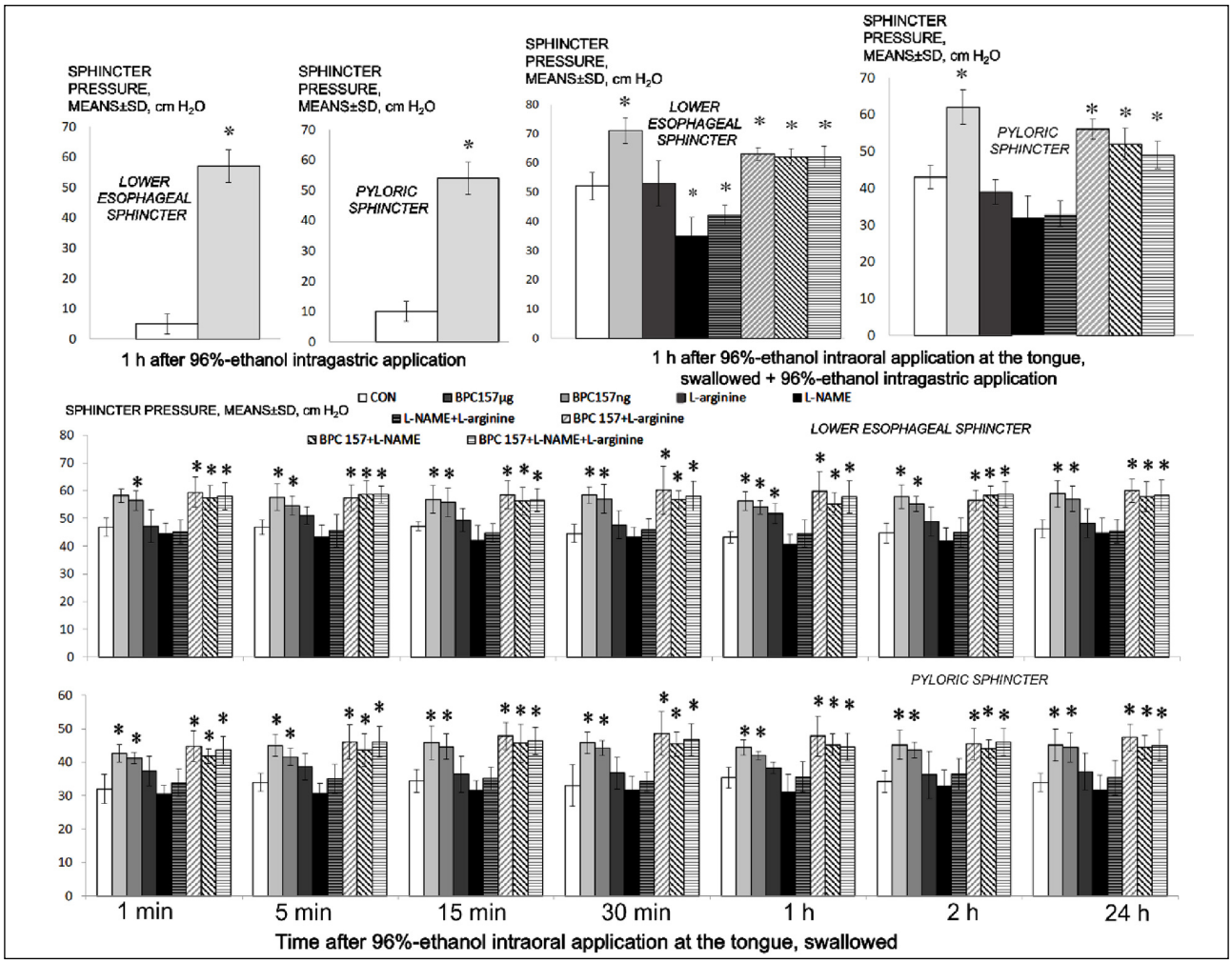

Fig. 4. Sphincter (lower esophageal and pyloric) pressure through 1 min-24 h after intra(per)-oral strong alcohol administration (1 ml/rat), swallowed, down, or, upper, at 1 hour: after intragastic strong alcohol administration (1 ml/rat) (*left*) or after combined administrations, intra(per)-oral strong alcohol administration (1 ml/rat) and swallowed + intragastic strong alcohol administration (1 ml/rat) (*right*). As described before (8, 14), in separate rats manometrical evaluation (cm H_2O) was performed before sacrifice with a water manometer connected to the drainage port of the Foley catheter as described (the values of 68 – 76 cm H_2O for lower esophageal sphincter and 68 – 74 cm H_2O for pyloric sphincter were considered to be normal). The proximal side of the esophageal or distal side of the duodenal incision was ligated to prevent regurgitation (8, 14). *Down*: Immediate post-alcohol intraperitoneal medication included pentadecapeptide BPC 157 (10 µg, 10 ng), L-arginine 100 mg, L-NAME 5 mg, given alone and/or together. Control received an equal volume of saline (5 ml/kg) immediately after strong alcohol and exhibited decreased pressure in either of sphincters. BPC 157 apparently rescued sphincters pressures, and maintained its effect also with NO-agents. NO-agents showed no effect. *Upper left*: Immediate post-alcohol intraperitoneal medication was pentadecapeptide BPC 157 (10 µg). Control received an equal volume of saline (5 ml/kg) immediately after strong alcohol. They presented minute pressure in both sphincters. BPC 157 apparently rescues pressure in both sphincters. *Upper right*: Immediate post-alcohol intraperitoneal medication (/kg) included pentadecapeptide BPC 157 (10 µg, L-arginine 100 mg, L-NAME 5 mg, given alone and/or together). Control received an equal volume of saline (5 ml/kg) immediately after strong alcohol, had decreased pressure in either of sphincters. BPC 157 apparently rescued sphincters pressures, and overrode NO-agents effects. NO-agents showed a limited NO-system involvement (in the lower esophageal sphincter (but not in the pyloric sphincter), L-arginine further decreased, L-NAME further decreased, while L-NAME+L-arginine rats presented values similar to those observed in controls). 10 rats/group at least, cm H_2O, means ± SD, *P < 0.05, at least versus control.

and striated muscle than BPC 157-rats (*Figs*. *7*, *8*). The histological patterns seen for drug regimens (*Fig*. *7*, *8*) correlated fully with the data from visual gross observation (*Fig*. *5*, *6*).

In other organs, including esophagus, stomach, and duodenum, mild edema of the lamina propria was commonly seen. After 24 h, the tongues of controls exhibited subepithelial focal accumulation of neutrophils, pronounced edema with abundant mastocytes in the striated muscle, and the area of macroscopic epithelial defects revealed a lack of surface epithelium with the defect covered by fibrinous exudate containing granulocytes and mononuclear inflammatory cells. On the contrary, BPC 157 treated animals presented a largely preserved surface epithelium with minor subepithelial foci of mononuclear inflammatory cells, and consistently less pronounced muscular edema with edema fluid appearing thicker, most likely containing more proteins (*Figs*. *7*(*B-5*), *10*, *11*).

Effect of ethanol intragastric administration on lesions in stomach, tongue, esophagus and duodenum.

The most severe lesions were noted in the stomach. Commonly, microscopic examination showed that in controls,

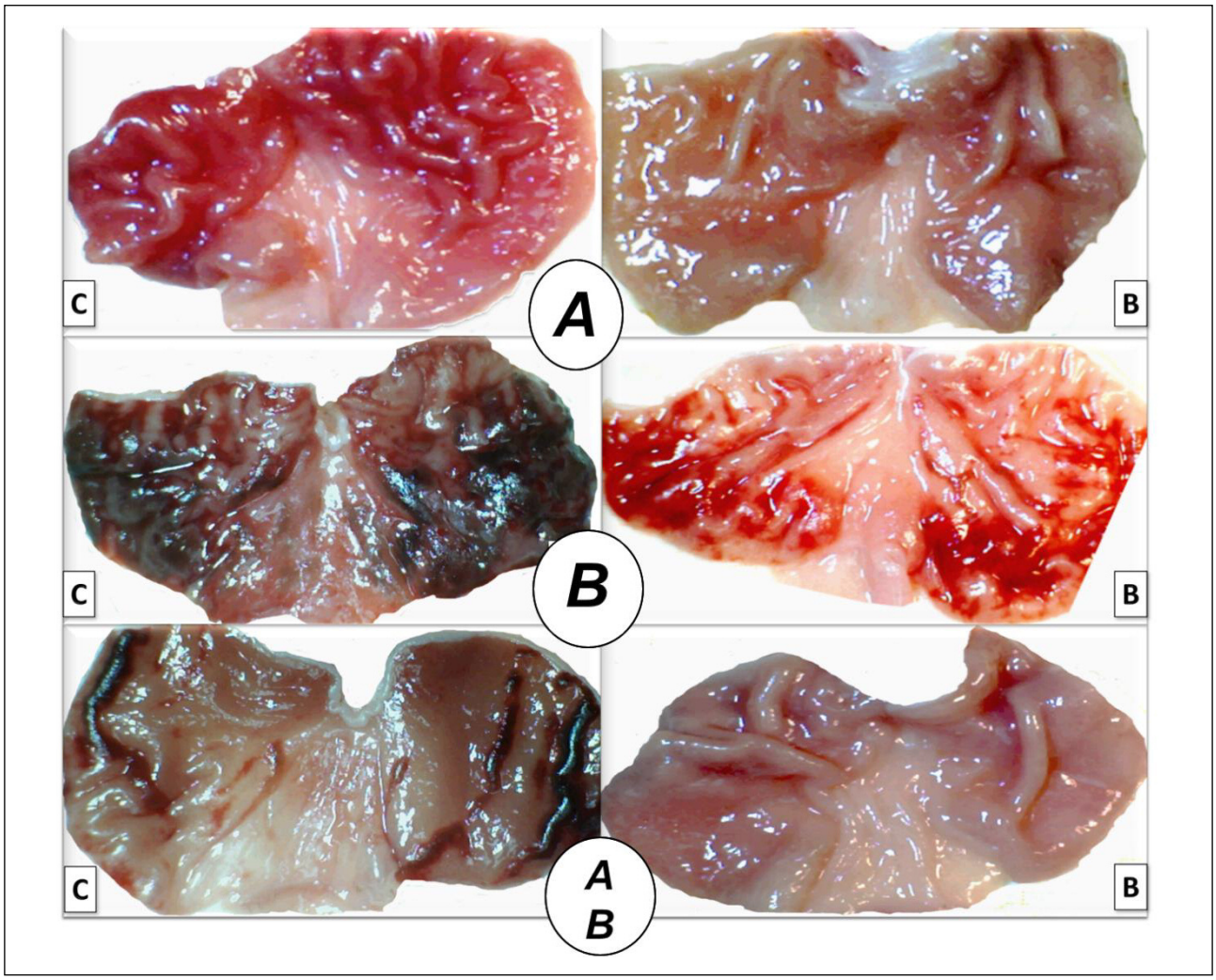

Fig. 5. Characteristic gross presentation of the lesions in the stomach glandular area in rats (Veho Discovery VMS-004 Deluxe USB microscope camera with its own light source) treated with BPC 157 (*B right*) and corresponding controls (*C left*). (*A*): After intra(per)-oral strong alcohol application, at the tongue, 1 ml/rat, and swallowed. (*B*): After intragastric strong alcohol application (1 ml/rat). (*AB*): After combined administrations intra(per)-oral strong alcohol administration (1 ml/rat), swallowed + intragastic strong alcohol administration (1 ml/rat). Presentation was at the 1 hour after strong ethanol administration.

necrotic areas involved the entire thickness of the gastric mucosa. There was regenerating activity on the surface, but the epithelium was not restored. This extensive epithelial destruction was confirmed by transmission electron microscopy. Many superficial epithelial cells showed various stages of degeneration with derangement of organelles and excessive mucus shedding. In animals treated with BPC 157, the pattern of injury differed from that in controls (*Figs. 5* and *6*). The surface epithelium showed less damage overall, during the entire experiment and most lesions did not penetrate further than the upper part of the gastric glands. Less congestion and hemorrhage were also obvious. Some areas of deep necrosis and submucous ulcers representing necrotic areas covered by intact mucosa were observed. Transmission electron microscopy also showed that, despite some degenerative changes in epithelial cells, the cohesion of the cells seemed to be much better preserved than in the controls. These results were also confirmed by scanning electron microscopy which, showed excessive basement membrane denudation in the controls, but not in the BPC 157 treated rats. The other tissues exhibited only mild lesions, *i.e.*, mild edema of the lamina propria irrespective to the given treatment.

Effect of combined administrations of ethanol (ethanol intra(per)-oral application, swallowed + ethanol intragastric application) on histology of the gastric mucosa.

In general, microscopic examination showed less extensive lesions than in those that received intragastric ethanol only. However, histologically, both in control and treated animals, erosions of different depth were visible. Some of these lesions showed different extents of hemorrhage not related to depth of the lesion. Macroscopically visible areas of hemorrhage, correlated well with histologically identified hemorrhagic lesions (*Fig. 8*).

DISCUSSION

This study reviewed a spontaneous reversal of strong alcohol-induced stomach lesions that occurs along with the application of strong alcohol at the rat tongue (the first target)-esophagus-stomach-duodenum chain of events, and lower esophageal and pyloric sphincter (mal)function. There is a therapy effect of the stable gastric pentadecapeptide BPC 157.

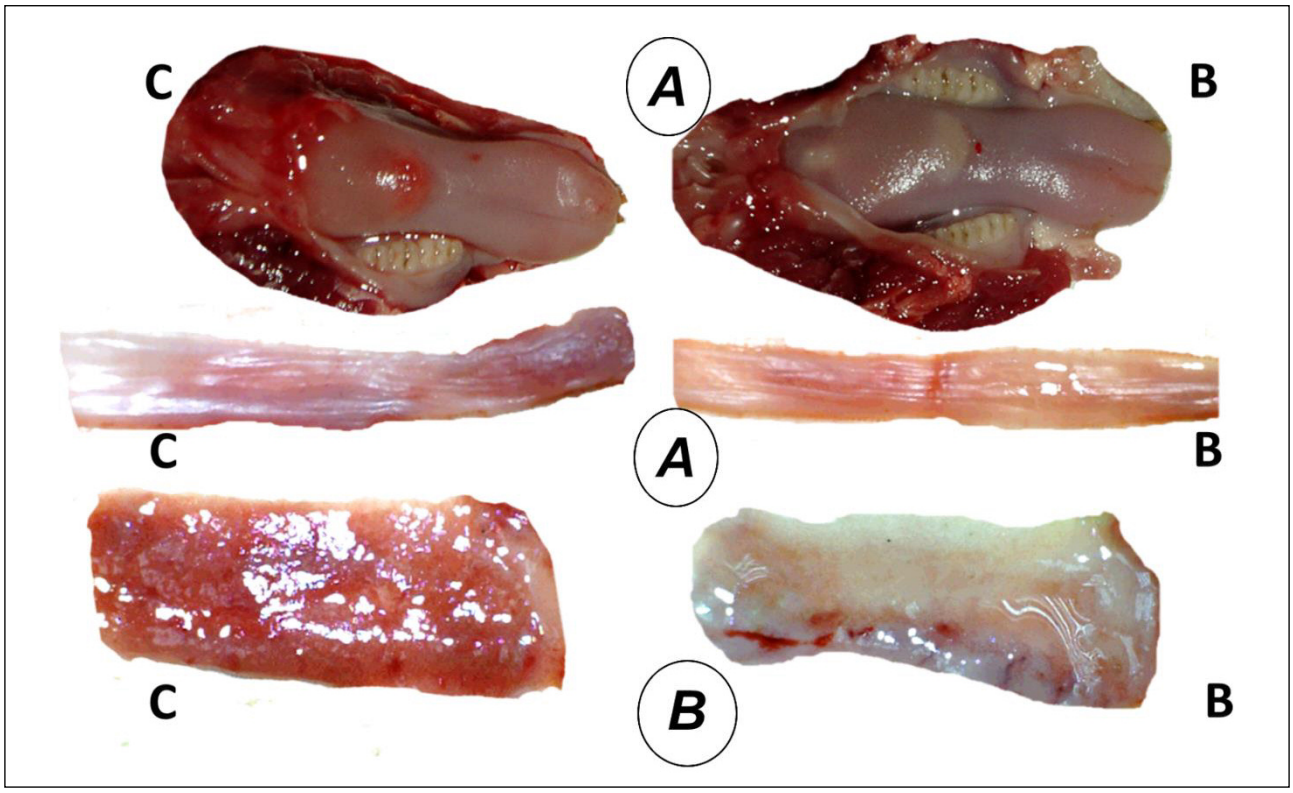

Fig. 6. Characteristic gross presentation of the lesions, redness, and congestion in the tongue (*upper*), proximal esophagus (*middle*) and duodenum (*lower*) in rats treated with BPC 157 (*B right*) and corresponding controls (*C left*). (*A*): After intra(per)-oral strong alcohol application, at the tongue, 1 ml/rat, and swallowed. (*B*): After intragastric strong alcohol application (1 ml/rat). Presentation was at 1 hour after strong ethanol administration.

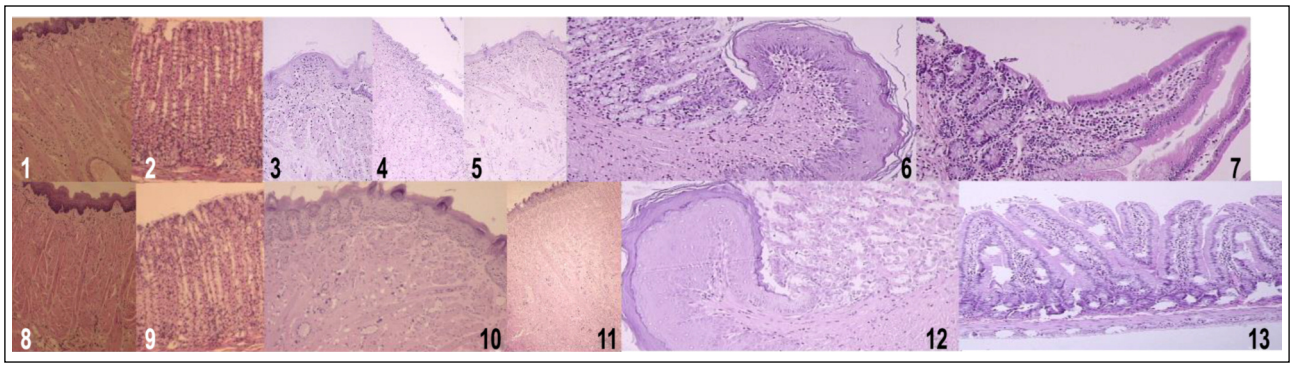

Fig. 7. Characteristic microscopic presentation at 2 h (1, 2, 8, 9) or 24 h (3 – 7, 10 – 13) after oral strong alcohol administration (1 ml/rat), at the tongue, and swallowed. 2 h (1, 2, 8, 9 white numbers), Tongue (1, 8): Mild reactive changes of the surface epithelium, scarce, unevenly distributed accumulations of po/ymorphonuclear inflammatory cells on the surface part of muscle. The controls exhibited more pronounced edema of stroma and striated muscle (1; ×10, H&E) than BPC 157-rats (8; ×10, H&E). Stomach (8, 9): Mild edema of the lamina propria in the stomach of control (8; ×10, H&E) and BPC 157-rats (9; ×10, H&E). 24 h, Tongue (3 – 5, 10, 11): Controls (3 – 5) exhibited subepithelial focal accumulation of neutrophils (3; ×10, H&E), the area of macroscopic epithelial defects histologically lacking the surface epithelium with the defect covered by fibrinous exudate containing granulocytes and mononuclear inflammatory cells (4; ×4, H&E). Pronounced edema with abundant mastocytes in tongue striated muscle controls (5; ×4, H&E). BPC 157-rats (10, 11) presented the surface epithelium largely preserved with minor subepithelial foci of mononuclear inflammatory cells (10; ×10, H&E), consistently less pronounced muscular edema (11; ×10, H&E). Esophagus (6, 12): Preserved superficial epithelium in the distal esophagus with more pronounced stromal edema and accumulations of polymorphonuclear inflammatory cells in controls (6; ×20, H&E) than in BPC 157-rats (12; ×20, H&E). Duodenum (7, 13): Proximal duodenum with preserved superficial epithelium, but more pronounced accumulations of polymorphonuclear inflammatory cells in controls (7; ×20, H&E) than in BPC 157-rats (13; ×10, H&E) was deserved.

With essential mitigation of the strong alcohol effect, we markedly renewed the functioning of the Robert's stomach cytoprotection/adaptive cytoprotection concept (1-3). No major stomach lesion appeared in any case of intra(per)-oral application 96%-ethanol at the tongue, that was swallowed, alone or with a subsequent 96%-ethanol intragastric application. Therefore, there was a complete overlapping cytoprotection/adaptive cytoprotection an effect that was considered physiologically

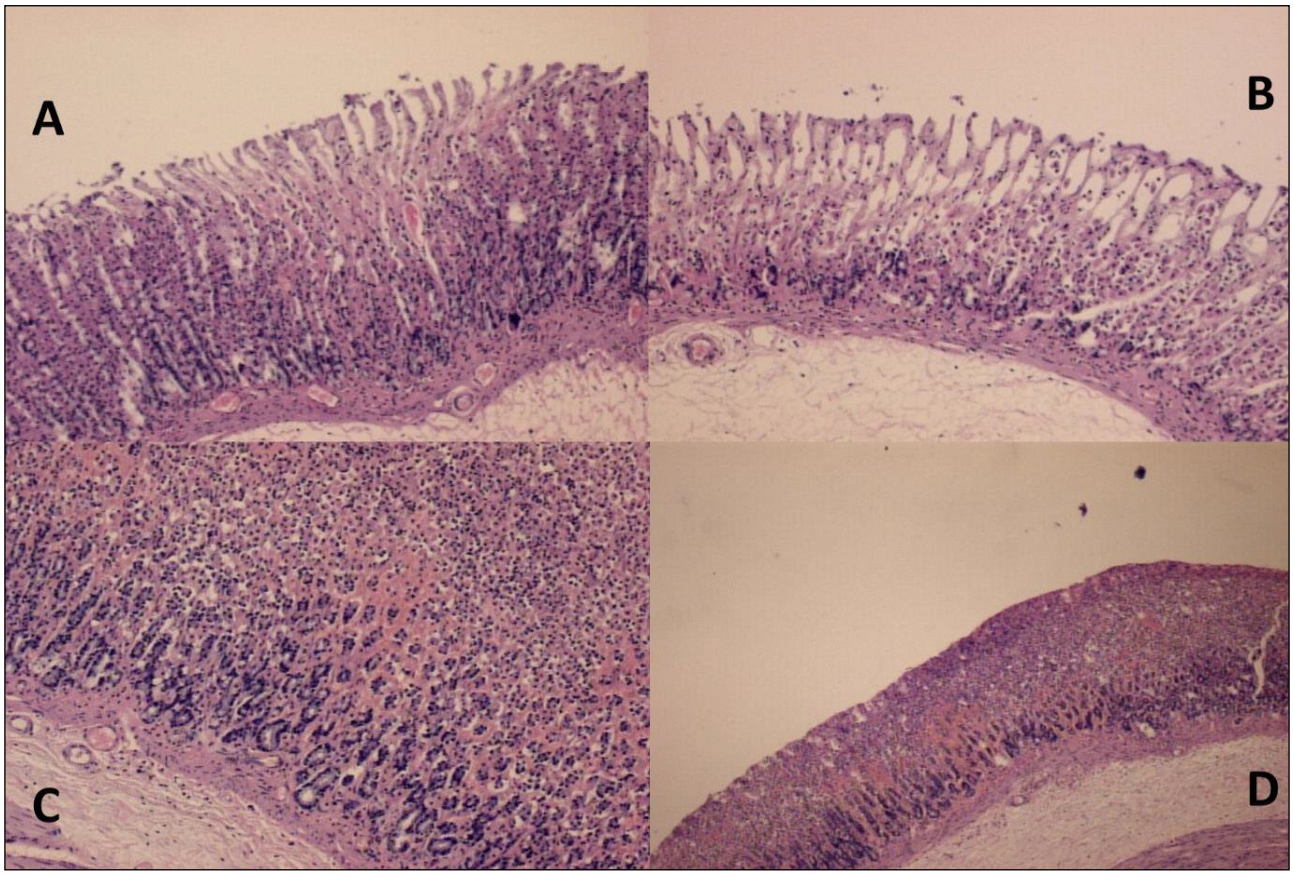

Fig. 8. Characteristic microscopic presentation at 1 h (A, B, C, D) after combined administration (intra(per)-oral strong alcohol administration (1 ml/rat), at the tongue, swallowed + intragastric strong alcohol administration 1 ml/rat). Superficial necrosis of gastric mucosa (A, ×4, H&E). Area of deep mucosal necrosis (B, ×4, H&E). Area of necrosis with scant, deep hemorrhage (C, ×4, H&E). Abundant hemorrhage with necrosis (D, ×2.5, H&E).

relevant. Always given after alcohol application, the therapy support should further amplify this newly revealed complete overlapping cytoprotection/adaptive cytoprotection. This should be the consistent beneficial effect of BPC 157 (4-16) given to pursue and amplify the effect on the already ongoing spontaneous defensive process.

Specifically, we demonstrated that intra(per)-oral application of 96%-ethanol at the tongue, swallowed, had a major impact at the tongue (histologically, mild reactive changes of the surface epithelium, scarce, unevenly distributed accumulations of polymorphonuclear inflammatory cells in surface part of muscle) while the controls exhibited more pronounced edema of stroma and striated muscle, than BPC 157-rats. Then the tongue, esophagus, and duodenum presented with extensive redness, while the stomach showed only minor lesions, an additional congestion in the whole of the glandular part with preserved mucosa. Accordingly, microscopy analysis showed in BPC 157 rats the stomach surface epithelium largely preserved with minor subepithelial foci of mononuclear inflammatory cells, the consistently less pronounced muscular edema with thicker edema fluid.

As mentioned, if only the minute stomach lesions appear, then the immediate overlapping cytoprotection/adaptive cytoprotection continuously operates in the stomach, as the next immediate application of the strong alcohol into the stomach was much more harmless. On the other hand, if the evidence of only minute stomach lesions with intraoral application at the tongue and swallowed, was not the immediate overlapping cytoprotection/adaptive cytoprotection continuously operating in the stomach, the stomach would have much more lesions from the double volume of the strong alcohol (alcohol at tongue, swallowed + alcohol intragastrically).

Likewise, an uphold resistance to alcohol is instantly present. If this was not the case, the strong alcohol with intraoral application at the tongue, swallowed, would induce the severe lesions in the stomach and not the small lesions as we noted.

Thus, the final positive outcome (mild lesion in the stomach) clearly indicated that the strong alcohol (administered at the tongue and swallowed; and then, also strong alcohol given intragastrically) consequently behaved as the mild alcohol. Likely, with regular eating/drinking, this indicates an always 'alerted' stomach that should be further basis also for the classic Robert's stomach cytoprotection/adaptive cytoprotection concept (1-3) functioning.

An additional supporting point, so far not noticed (1-3), belongs to the lower esophageal and pyloric sphincter function. The small lesions in the stomach with strong alcohol intra(per)-oral application at the tongue, swallowed, went with considerable pressure decrease in the lower esophageal and pyloric sphincter. Even more, classic strong alcohol intragastric application induced severe stomach lesions with complete pressure decrease in lower esophageal and pyloric sphincter (and thereby, lesions appear in the esophagus and tongue as well as in duodenum). Therefore, it may be an especial alcohol mucosal lesions-sphincters failure relation. Also, this relation raises cause-consequence question, particularly since BPC 157 can distinctively rescue failure of the lower esophageal and pyloric sphincter (34-42), as well as other sphincters failures (43, 44).

A common beneficial link may be the already known therapy effect of BPC 157 given after strong alcohol intragastric application (13, 30-33). Previously, such full therapy effect overrides the effect of standard cytoprotective agents (which is only prophylactic and not therapy effect) (1-3), and results in its full implication in stomach cytoprotection and adaptive cytoprotection, as a representative of new class of cytoprotective agents (13, 30-33). In the same way, given after, BPC 157 can ameliorate the original beneficial course after intra(per)-oral application of 96%-ethanol at the tongue, swallowed, (and then the tongue, esophagus, and duodenum presented with extensive redness, and in particular, those minor lesions in the stomach all markedly further improved). Likewise, it ameliorates the additional course after subsequent immediate intragastric application of 96%-ethanol. Also, as mentioned, it rescued the pressure fall in the lower esophageal and pyloric sphincter whatever it appeared after intra(per)-oral application of strong alcohol at the tongue, swallowed, without or with the immediate subsequent intragastric application of strong alcohol as well as after classic intragastric application of strong alcohol. The combined therapy effect involving both mucosal and sphincter function may be essential. Since BPC 157 rescued sphincter functions after various damaging events (30-40), this suggests a key role for the sphincters less or more damaged, and thereby less or more malfunction, less or more damage development.

Extension to the NO studies of the novel phenomenon following intra(per)-oral strong alcohol administration and investigation under complex NO-conditions appears to be a rather logical approach. To this point we used a blunted generation of NO-pathway (NOS-blocker, L-NAME), an (over)-stimulated NO-system (NOS-substrate, L-arginine), and NO-system immobilized (in which L-NAME + L-arginine would antagonize each other's responses to confirm specific NO-system involvement) (10, 45, 46). Gastric pentadecapeptide BPC 157 application interacts with NO-system in various models and species and NO-system is essentially important signaling system in the gastrointestinal tract (6, 10). Specifically, the NO-specific effects of NO-agents *i.e*., beneficial effect for L-arginine, ulcerogenic for NO generation inhibitors (6, 10, 45, 46) in cytoprotection/adaptive cytoprotection systems are based on the relationship observed after the intragastric instillation of the strong alcohol to the stomach (31). There, the huge gastric lesions after intragastric alcohol appeared as a maximal, almost definitive failure of the NO-system *i.e*., the additional NOS-blockade (L-NAME) could not further aggravate these lesions; however, L-NAME did antagonize the L-arginine beneficial effect (31). By contrast with the intra(per)-oral application of the strong alcohol, the innate effect of L-NAME and additional NOS-blockade existed with the intra(per)-oral application of the strong alcohol (tongue lesions aggravated), and further, with additional subsequent immediate intragastric application of the strong alcohol (tongue and esophagus lesions were aggravated), thus, a non-definitive NO-system failure was quickly initiated. This point supports the opposite effect of L-arginine and in addition, the evidence that when given together, L-NAME + L-arginine could antagonize each other's response. However, these L-NAME effects would not affect the stomach lesions presentation likely because of the process of overlapping cytoprotection/adaptive cytoprotection that was immediately initiated. Likewise, with NO-agents it could be not established that the effect on mucosal presentation was extended to a comparable sphincter function. Illustratively, only after combined strong alcohol administration did NO-agents show some effects suggesting a particular NO-system involvement. Pressure in lower esophageal sphincter (but not in pyloric sphincter) L-arginine further decreased, L-NAME also further decreased while L-NAME + L-arginine rats presented similar results as controls. Thus, these facts limited significance of the NO-system involvement.

Finally, the administration of the stable gastric pentadecapeptide BPC 157 overwhelmed all of these NO-agents effects. BPC 157, being able to better antagonize NOS-blockade than L-arginine did (6, 10, 31), effectively reached the higher level of rescue, and reinstated sphincter function. In studies using homogenate supernatants of rat gastric mucosa, the pentadecapeptide BPC 157 had an effect on NO generation that was more pronounced and/or different from the effect of L-arginine (31, 47).

In conclusion, there is a continuous debate about cytoprotection (1-3) and significance of the cytoprotection to the mechanism of gastric mucosal integrity and defense as well as ulcer healing (48-56). Thereby, the even more importance belongs to the novel evidence that there is a spontaneous mitigation of the strong alcohol effect and the involvement of a potent vasodilators H_2S and CO in machanism of gastroprotection (52-56). Taking the stomach minute lesions presentation as the outcome, we offer novel evidence for how cytoprotection/adaptive cytoprotection instantly functions in the stomach from tongue (first target), and then esophagus-stomach-duodenum chain of events, and impaired lower esophageal and pyloric sphincter. The challenge was 96%-ethanol intra(per)-orally at the tongue and swallowed, without or with additional intragastric strong ethanol application. Given after alcohol, stable gastric pentadecapeptide BPC 157 interfered with the already advanced process, and augmented the complete defensive process, mucosal and sphincter function. This supports its relevance in the cytoprotection/adaptive cytoprotection course, and possible use in therapy (4-16). On the other hand, L-arginine and L-NAME administration can affect the process partially, having no major influence on final outcome, lesions in the stomach or sphincters function.

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