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Z. BILIC[1], S. GOJKOVIC[1], L. KALOGJERA[1], I. KREZIC[1], D. MALEKINUSIC[1], M. KNEZEVIC[1], M. SEVER[1], N. LOJO[1], A. KOKOT[2], K. KASNIK[1], T. KRALJ[1], J. VUKOJEVIC[1], M. SIROGLAVIC[1], M. PEKLIC[1], D. DRMIC[1], M. MILAVIC[3], S. SIKIRIC[3], I. SKORAK[1], I. BRIZIC[1], K. HRIBERSKI[1], M. KUBAT[4], J. VLADIC[5], A. BOBAN BLAGAIC[1], A. TVRDEIC[1], A. SKRTIC[3], S. SEIWERTH[3], P. SIKIRIC[1]

NOVEL INSIGHT INTO ROBERT'S CYTOPROTECTION: COMPLEX THERAPEUTIC EFFECT OF CYTOPROTECTIVE PENTADECAPEPTIDE BPC 157 IN RATS WITH PERFORATED STOMACH THROUGHOUT MODULATION OF NITRIC OXIDE-SYSTEM. COMPARISON WITH L-ARGININE, RANITIDINE AND PANTOPRAZOLE THERAPY AND L-N^G-NITRO-L-ARGININE METHYL ESTER WORSENING

[1]Department of Pharmacology, School of Medicine, University of Zagreb, Zagreb, Croatia; [2]Department of Anatomy and Neuroscience, Faculty of Medicine, J.J. Strossmayer University of Osijek, Osijek, Croatia; [3]Department of Pathology, School of Medicine, University of Zagreb, Zagreb, Croatia; [4]Department of Forensic Medicine and Criminology, School of Medicne, Zagreb, Croatia, [5]Laboratory for Advanced Genomics, Division of Molecular Medicine, Institute Ruder Boskovic, Zagreb, Croatia

Surgically perforated stomach (since direct injury in rats until persisting defect and huge adhesions (day 1, day 7)) fairly represent an unresolved cytoprotection issue, and thereby, we focused resolving of the immediate triad, particular vascular failure (vessels 'disappear'/empty), prolonged bleeding, debilitated defect large widening. Agents (mg/kg) or saline (controls) were given at 1 min post-injury as an abdominal bath (10 ml/rat throughout 2 min). Within 1 – 15 min post-injury period, with cytoprotective BPC 157 (0.01 μg), the rapidly restored vessels 'run' (vessels filled/reappeared) toward the perforated defect, and there is less bleeding, and defect contraction; advanced perforated lesion healing (day 1) to complete healing (day 7), and less adhesions. With pantoprazole (10 mg), early (vessels (worsening), bleeding (prolongation), defect (attenuated widening)) effect means eventual lesions and adhesions severity as in controls. Ranitidine (10 mg) early effect (vessels (improvement), bleeding (less bleeding), defect (eliminated widening, defect not changed)) means final lesions attenuation, but not complete healing, less adhesions. L-NAME (5 mg) early (vessels worsening, less bleeding, attenuated defect widening) and final (lesions aggravation, more adhesions) effect, versus L-arginine (100 mg) early (vessels improvement, more bleeding, attenuated defect widening) and final (lesions attenuation, less adhesions) effect, combined few simultaneously occurring nitric oxide (NO)-system distinct processes. Finally, in the stomach tissue surrounding defect, increased malondialdehyde (MDA)- and decreased NO-values, BPC 157 reversed to the normal healthy values, and mRNA expression studies (*Cox2*, *VEGFa*, *Nos1*, *Nos 2*, *Nos3*, *Nkap* (NF-kappa-B-activating protein gene)), done at that very early post-perforation-time, indicate a way how BPC 157 may act beneficially in the perforated stomach lesion throughout NO- and prostaglandinds-system.

Key words: *Pentadecapeptide BPC 157, nitric oxide vessels, perforated stomach, L-arginine, L-N^G-Nitro-L-arginine methylester, pantoprazole, ranitidine, cytoprotection, cyclooxygenase 2, bleeding time*

INTRODUCTION

Interestingly from the cytoprotection perspective, the perforation of the stomach (1-3), as the prototype lesion made by a direct injury, remained so far not included in the cytoprotection studies (4, 5), and thereby, outside of the resolving possibility for the new therapy achievements as well (6).

Recently, we reviewed particular upgrading of the classic cytoprotection concept (7), years ago made by Robert (various noxious agents, direct injury to cell, epithelium protection/maintenance) (4) and Szabo (endothelium protection/maintenance) (5, 8) and their synergistically working axiom (endothelium maintenance › epithelium maintenance) (7), which had been overwhelmingly focused on the stomach cytoprotection (4, 5, 8). On the other hand, the upgrading of the concept implied the greater pleiotropic cytoprotection potential of the stable gastric pentadecapeptide BPC 157 (7), native and stable in human gastric juice, as novel cytoprotection mediator largely involved in the wound healing (7, 9). Here, with BPC 157 therapy, we attempt to resolve in the rats with the perforated stomach, the immediate post-perforation threat, as an essential cytotoxic/cytoprotective event. There are the initial failure and

recovery of the vessels, and more or less bleeding upon perforation, and lesion presentation (initial defect widening upon injury or shrinking down upon drug application), and then, the subsequent healing in the forthcoming days, as the special target. To resolve this issue, we especially appraised its novel very specific therapy effect on damaged vessels (for review see (7)). This relayed also on the counteraction of the consequent multiorgan failure, as a part of the major vessels occlusion, occlusion-syndrome (10-18), as well as occlusion like-syndrome (*i.e.* major intoxication *e.g.* alcohol, lithium), maintained intra-abdominal hypertension- and myocardial infarction-induced major vessel failure (19-22) and Virchow triad circumstances that may be commonly presented (7).

Also, there is a supporting evidence in the rats with the perforated cecum (23). The rapidly restored vessels 'run' (vessels filled/reappeared) toward the defect at the perforated lesion with BPC 157 therapy, which may have distinct clinical strategies for the open bleeding and to heal the stomach lesions (23), as a particular target in the cytoprotection studies (4-7), but now with interconnected pathology that likely argues the common solution. There, it may be challenged the significance of nitric oxide (NO)-system in the gastrointestinal lesions healing (24-29), the endothelium lesions and bleeding disorders (30-32), with N^G-nitro-L-arginine methylester (L-NAME), NO-synthase (NOS)-blockade pro-thrombotic effect or L-arginine (NOS-substrate) anti-thrombotic effect, which would antagonize each other effect if NO-system related, given alone or together, or combined with BPC 157 (33). Ranitidine and pantoprazole were standard agents.

Indicatively, there is quite strong evidence for the BPC 157 maintaining of the prostaglandins-system (34), which function is essential in the cytoprotection concept (4-8) and in the BPC 157 cytoprotective effectiveness (35). BPC 157 largely antagonizes all alcohol-induced lesions (19, 29, 36-42) and counteracts nonsteroidal anti-inflammatory drugs (NSAIDs)-induced adverse effects (43-51), including leaky gut (51). The therapy effect includes nonspecific NSAIDs (43-49, 51) and specific NSAID (50), in particular, curing stomach lesions that regularly were perforated or about to perforate (34). Furthermore, important for the perforated lesions healing, there is the fistulas healing, as 'two sides' wounds (52). Namely, BPC 157 healed also the perforated lesions created in the different tissues which suturing together provided various fistulas, and thereby particular healing evidence for the both external (53-56) and internal (57-59) fistula resolving. Even more, BPC 157 modulates NO-system (30) as without the need for other known ligands or shear stress, BPC 157 activates the VEGFR2-Akt-eNOS signaling pathway (60), and maintains the vasomotor tone through the activation of Src-Caveolin-1-eNOS pathway (61). Accordingly, BPC 157 induced the NO release on its own, resistant to L-NAME application (29, 62), abolished in the same way both the NOS blocker L-NAME-induced hypertension and pro-thrombotic effect and the NOS substrate L-arginine-induced hypotension and anti-thrombotic effect (29, 33). Also, BPC 157 maintained thrombocytes function without affecting coagulation pathways and counteracted prolonged bleeding in rats with tail or leg amputation (47), cecum perforation (23), or prolonged deep veins thrombosis (10).

Thus, we suggest the perforated stomach as heretofore-undiscovered pathway in the Robert's stomach cytoprotection concept (4, 7). Note, the evidence that the standard cytoprotective agents are effective only given before injury (*i.e.*, Robert's prostaglandins), but not after (4, 7), precludes their use in the perforated stomach healing.

Thereby, we used the essential advantage of BPC 157 application, therapy effect, and as in other studies (7, 30, 34, 35, 63-74), we have started to administer the regimen after the wound has been created, providing Robert's original claim that the cytoprotective agents should induce reversal of the damaged tissue to normal structure, interruption of the damaging events (4). To further clarify and show a direct beneficial effect, starting with rapid vessels recovery since the initial injurious event, medication (BPC 157, L-NAME, and L-arginine, alone and/or together; ranitidine, pantoprazole) was administered directly to the injury (23, 75, 76) once, during surgery *via* a medicated bath to the perforated stomach (then, the solution spreads through the abdominal cavity). At that very early point, we carried out the oxidative stress and NO-determination in the stomach tissue. Likewise, a particular gene expression (*i.e.*, cyclooxygenase *(Cox)2*, vascular endothelial growth factor *(VEGF)a*, *Nos1*, *Nos2*, *Nos3*, *Nkap* (NF-kappa-B-activating protein gene)) (10, 18), was carried out as a likely special point to explain how the dysfunction and its counteraction is causal to, or result of. Thereby, having a very safe profile (lethal dose (LD1) not achieved), implemented in inflammatory bowel disease trials, as recently reviewed (7, 30, 34, 35, 63-74), BPC 157 should be the practical hallmark of the further cytoprotection healing therapy in the emergency situation such perforated stomach lesion.

MATERIALS AND METHODS

Animals

The study protocols were conducted in male Albino Wistar laboratory rats with body weights of 200 g. The rats were 13 weeks old and bred in-house at the Pharmacology Department of the School of Medicine, University of Zagreb, Zagreb, Croatia animal facility. This animal facility is registered by the Directorate of Veterinary Services (Reg. No: HR-POK-007). The laboratory rats were acclimated for 5 days and randomly assigned to their respective treatment groups. The laboratory animals were housed in PC cages in conventional laboratory conditions at a temperature of 20 – 24°C, a relative humidity of 40 – 70% and noise level of 60 DCB. Each cage was identified according to the following: the number of the study, the group, the dose, and the number and sex of each animal. Fluorescent lighting provided illumination for 12 hours per day. A standard good laboratory practices (GLP) diet and fresh water were provided *ad libitum*.

The animal care complied with the standard operating procedures (SOPs) of the Pharmacology Animal Facility and the European Conventions for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS 123). The ethical principles of the study ensured compliance with the European Directive 010/63/E, the Law on Amendments to the Animal Protection Act (Official Gazette 37/13 and the Animal Protection Act, Official Gazette 135/06), the Ordinance on the protection of animals used for scientific purposes (Official Gazette 55/13), the FELASA recommendations and the recommendations of the Ethics Committee of the School of Medicine of the University of Zagreb. All experiments received specific approval from the Local Ethics Committee at the School of Medicine of the University of Zagreb, Zagreb, Croatia.

We randomly assigned at least 6 rats to each experimental group and period for all experiments. The perforation procedure was performed in rats with *ad libitum* food and water access before the procedure and until the end of the experiment, and the procedure was assessed by an observer who was unaware of the treatment.

Drugs

Pentadecapeptide Gly-Glu-Pro-Pro-Pro-Gly-Lys-Pro-Ala-Asp-Asp-Ala-Gly-Leu-Val, M.W. 1419, *i.e.*, BPC 157, which is a part of the sequence of the human gastric juice protein that

encodes BPC, was prepared by dissolution in water at pH 7.0 and in saline (Diagen, Ljubljana, Slovenia) as described previously (7, 30, 34, 35, 63-74). The peptide exhibited 99% purity *via* high-pressure liquid chromatography (HPLC); the 1-des-Gly peptide was detected as an impurity, but this compound is biologically inactive (7, 30, 34, 35, 63-74). Pantoprazole, ranitidine, L-NAME and L-arginine were commercially purchased (Sigma Aldrich, St. Louis, MO, USA) and dissolved in saline.

Perforated defect creation, medication, and assessment

In deeply anesthetized rats (intraperitoneally applied 40 mg/kg thiopental (Rotexmedica, Trittau, Germany) and 10 mg/kg diazepam (Apaurin; Krka, Novo Mesto, Slovenia)), the stomach was exposed, and a perforation was created with a 5-mm diameter metal needle on the ventral side in the prepyloric area. The rats were monitored for the next 15 minutes and at the following specific time points, indicated as A, B, C, D, and E: A, after perforation (1 minute); B, during application (2 minutes); C, the period after application (2 minutes); D, the next 5-minute period; and E, the period until the end of the observation (15 minutes).

Based on the previous studies (for review see (7, 30, 34, 35, 63-74)), the medications (/kg) included BPC 157 (10 µg), the NOS-blocker L-NAME (5 mg), and the NOS substrate L-arginine (100 mg), alone or in combination, as well as the proton pump inhibitor pantoprazole (10 mg), the H_2-blocker ranitidine (10 mg), and an equal-volume saline bath (controls). Note, at 1-minute post-injury, the medication was directly applied to the perforation site (5 mm diameter) of the prepyloric lesion and stomach, and spread through the abdominal cavity, as described before (23), as a bath, 10 ml/2 minute bath/rat, as follows: (i) in combination studies, 5 ml of the each of the two agents, or 3.3 ml of the each of the three agents, given simultaneously; (ii) in separate agent application, with agent's 5 ml volume, additional 5 ml of saline, given simultaneously; (iii) 10 ml of saline (controls). The rats were left undisturbed after abdominal closure until sacrifice.

Using previous procedure (10-22), assessments were performed with a USB microscope camera (Veho Discovery VMS-004 Deluxe). We recorded and assessed the blood vessels in terms of whether they were filled (appearance) or emptied (disappearance) at areas close to the perforated lesion or in the remaining stomach, distant from the perforated lesion. We also assessed defect closing or widening (both as % of the presentation immediately before therapy) at all the indicated time points until the end of the 15-minute period. The bleeding time from the perforation (s) was assessed throughout the 15 min, as described (10, 23, 33, 47).

Next, at day 1 and the final point at day 7, the defect sizes (*i.e.*, serosal and mucosal; largest lesion diameter, mm) and adhesion severity (77) (scored 1 – 9; specifically, adhesions of the mesentery and intestine) were assessed, with the adhesion scores given as not present (score 0) or present (score 1); adhesions of the liver/spleen were scored 0 – 3 (score 0: no adhesions; score 1: adhesions occupying less than one-third of the organ surface; score 2: more than one-third but less than two-thirds; score 3: more than two-thirds). Thus, the total maximum score was 8; visible perforations were scored as 1, and the worst score was 9.

Representative tissues sections were processed for further histological analysis as described previously (10-22).

Oxidative stress

At 15 minutes post-injury, oxidative stress in the stomach tissue samples was assessed by quantifying the thiobarbituric acid (TBA) reactivity as malondialdehyde equivalents (MDA). To homogenize the tissue samples, trichloroacetic acid (TCA) was added, the samples were centrifuged (3000 rpm, 5 min), and the supernatants were collected. Thereafter, 1% TBA was added, and the samples were boiled (95°C, 60 min). The tubes were kept in ice for 10 min, and the absorbance was determined at the wavelengths of 532 and 570 nm. The concentration of MDA was read from the standard calibration curve, which was plotted using 1,1,3,3'-tetra-ethoxy propane (TEP). The extent of lipid peroxidation is expressed as the concentration of MDA, using a molar extinction coefficient for MDA of 1.56 × 105 mol/L/cm. The results are expressed in nmol/mg of protein.

Nitric oxide determination

At 15 minutes post-injury, we determined the nitric oxide (NO) levels in the stomach tissue samples using the Griess reaction (Griess Reagent System, Promega, Madison, WI, USA). Sulfanilamide was added to the homogenized tissue, which was incubated, and then, N-(1-naphthyl)ethylenediamine dihydrochloride was added. The Griess reaction is based on the diazotization reaction in which acidified nitrite reacts with diazonium ions and, in a further step, is coupled to N-(1-naphthyl) ethylenediamine dihydrochloride to form a chromophoric azo derivative. The absorbance was measured at 540 nm using a sodium nitrite solution as the standard. The NO levels are reported in µmol/mg protein. The proteins were determined using a commercial kit (BioRad Protein DR Assay Reagent Kit, BioRad Labs., Hercules, CA, USA).

Gene expression analysis

Using procedure as before (10, 18), after sacrifice at 2, 5, and 15 min after application of the saline (5 ml/kg) or BPC 157 (10 ng/kg), tissue (stomach tissue, 1 mm around the defects) was rapidly dissected and frozen in liquid nitrogen. The tissue was disrupted using tissue homogenizer Bio-Gen PRO200 homogenizer (PRO Scientific, Oxford, CT, USA), in 1000 µl of TRIzol (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) and the isolation itself was done using a TRIzol-based reagent method according to the manufacturer's instructions.

After RNA isolation step, nucleic acid concentration was measured with Nano Drop ND-1000 spectrophotometer (Nano DropTechnologies, Thermo Fisher Scientific, Waltham, MA, USA).

Reverse transcription was performed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) following manufacturer's instructions and using a ProFlex PCR System machine (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA).

TaqMan Gene Expression Assay (Applied Biosystems, Termo Fisher Scientific, Waltham, MA, USA) hydrolysis probes were used for gene expression analysis of selected genes (*Table 1*) with TaqMan Gene Expression Master Mix (Applied Biosystems, Termo Fisher Scientific, Waltham, MA, USA). Quantitative PCR was carried out in duplicate for every sample. Reactions were performed with Cobas z 480 instrument (Hoffmann-La Roche Ltd, Basel, Switzerland) according to the following protocol: 2 min at 50°C, 10 min at 95°C, 45 cycles of 15 s at 95°C and 1 min at 60°C.

We analyzed *Actb* as reference gene to normalize results of several genes of interest: *Cox2*, *Vegfa*, *Nos1*, *Nos2*, *Nos3* and *Nkap*.

To determine the difference in gene expression between treated and non-treated samples, the formula 2-ΔΔCt was used, where the ΔΔCt is the difference between ΔCt of the treated sample and the ΔCt of the non-treated sample (*Table 1*).

Table 1. Selected genes details and TaqMan Assays specifications.

Gene symbol	Synonyms	Gene name	TaqMan Assay ID	NCBI Reference Sequence	Amplicon length (bp)
Actb		Actin, beta	Rn00667869_m1	NM_031144.3	91
Nkap	2610020o08rik	NFκB activating protein	Rn01297610_m1	NM_001024872.1	87
Nos1	nNOS, bNOS	Nitric oxide synthase 1	Rn00583793_m1	NM_052799.1	65
Nos2	iNos, Nos2a	Nitric oxide synthase 2	Rn00561646_m1	NM_012611.3	77
Nos3	cNOS, eNos	Nitric oxide synthase 3	Rn02132634_s1	NM_021838.2	117
Ptgs2	COX-2, Cox2, cyclooxygenase 2	Prostaglandin-endoperoxide synthase 2	Rn01483828_m1	NM_017232.3	112
Vegfa	VEGF-A, VPF	Vascular endothelial growth factor A	Rn01511601_m1	NM_001110333.2	69

Table 2. Assessed stomach lesion (sum of longest diameters, mm, means ± SD, at mucosal (M) and serosal side (S)) and adhesion severity (score 0 – 9, Min/Med/Max). *$P < 0.05$, at least vs. control.

Medication (/kg, 10 ml/2 min bath/rat)	**Assessed stomach lesion** (sum of longest diameters, mm, means ± SD) **and adhesion severity** (score 0 – 9, Min/Med/Max)			
	Day 1		Day 7	
	Perforate stomach lesion	**Adhesion severity**	**Perforate stomach lesion**	**Adhesion severity**
0.9%NaCl (control)	5.0 ± 0.5 (M) 5.0 ± 0.5 (S)	4/5/5	5.0 ± 0.5 (M) 3.0 ± 0.5 (S)	8/9/9
BPC 157 (10 µg)	*3.0 ± 0.0* (M)* *3.0 ± 0.0* (S)*	*1/1/1**	*0.0 ± 0.0* (M)* *0.0 ± 0.0* (S)*	*2/3/3**
L-NAME (5 mg)	*7.2 ± 0.4* (M)* *7.0 ± 0.5* (S)*	*6/6/6**	*7.0 ± 0.4* (M)* *5.0 ± 0.5* (S)*	8/9/9
L-arginine (100 mg)	*3.0 ± 0.0* (M)* 5.0 ± 0.0 (S)	*2/3/3**	*2.0 ± 0.0* (M)* *0.0 ± 0.0* (S)*	*5/6/7**
L-NAME (5 mg) + **L-arginine** (100 mg)	5.0 ± 0.5 (M) 5.0 ± 0.5 (S)	4/5/5	4.0 ± 1.3 (M) 3.0 ± 0.5 (S)	8/8/9
L-NAME (5 mg) + **BPC 157** (10 µg)	*3.0 ± 0.0* (M)* *3.0 ± 0.0* (S)*	*1/1/2**	*0.0 ± 0.0* (M)* *0.0 ± 0.0* (S)*	*4/4/5**
L-arginine (100 mg) + **BPC 157** (10 µg)	*3.0 ± 0.0* (M)* *3.0 ± 0.0* (S)*	*1/1/2**	*0.0 ± 0.0* (M)* *0.0 ± 0.0* (S)*	*4/4/5**
L-NAME (5 mg) + **L-arginine** (100 mg) + **BPC 157** (10 µg)	*3.0 ± 0.0* (M)* *3.0 ± 0.0* (S)*	*1/1/2**	*0.0 ± 0.0* (M)* *0.0 ± 0.0* (S)*	*2/3/3**
Pantoprazole (10 mg)	5.0 ± 0.5 (M) 5.0 ± 0.5 (S)	4/4/5	4.5 ± 0.5 (M) 2.5 ± 0.5 (S)	8/9/9
Ranitidine (10 mg)	*3.5 ± 0.4* (M)* 5.0 ± 0.5 (S)	*2/3/3**	*2.5 ± 0.4* (M)* *0.0 ± 0.0* (S)*	*5/6/6**

Statistical analysis

The statistical analyses were performed with parametric one-way ANOVAs with *post hoc* Newman-Keuls tests and non-parametric Kruskal-Wallis tests with subsequent Mann-Whitney U tests to compare groups. The values are represented as the mean ± SD as well as the minimum, median, and maximum. The results were considered significant at $P < 0.05$.

RESULTS

In the rats with the perforated stomach, the initial focus was on the particular early post-injury period (*Figs*. *1-8*), with the cytoprotective stable gastric pentadecapeptide BPC 157, L-NAME, L-arginine, NO-system relation vs. standard H2 blocker or proton pump inhibitor. We attempt to reveal the stomach perforation early healing (*Figs*. *1-8*) and course throughout the next 7 days (*Table 2*, *Fig*. *9*), and therapy effect upon conditions of the once time medication at very early post-injury period. Upon perforation injury, there is a so far poorly described early cluster (vessels, bleeding, defect), which may be essential for the final effect (perforated defect healing, adhesions formation).

Regularly, the control rats presented the vessel failure (vessels 'disappear'/empty, both veins and arteries) (*Figs*. *1*, *2*, *5* and *6*). With therapy, when successful, the vessels recovery (the rapidly restored vessels 'run' (vessels filled/reappeared) toward the defect at the perforated stomach), as noted in BPC 157-

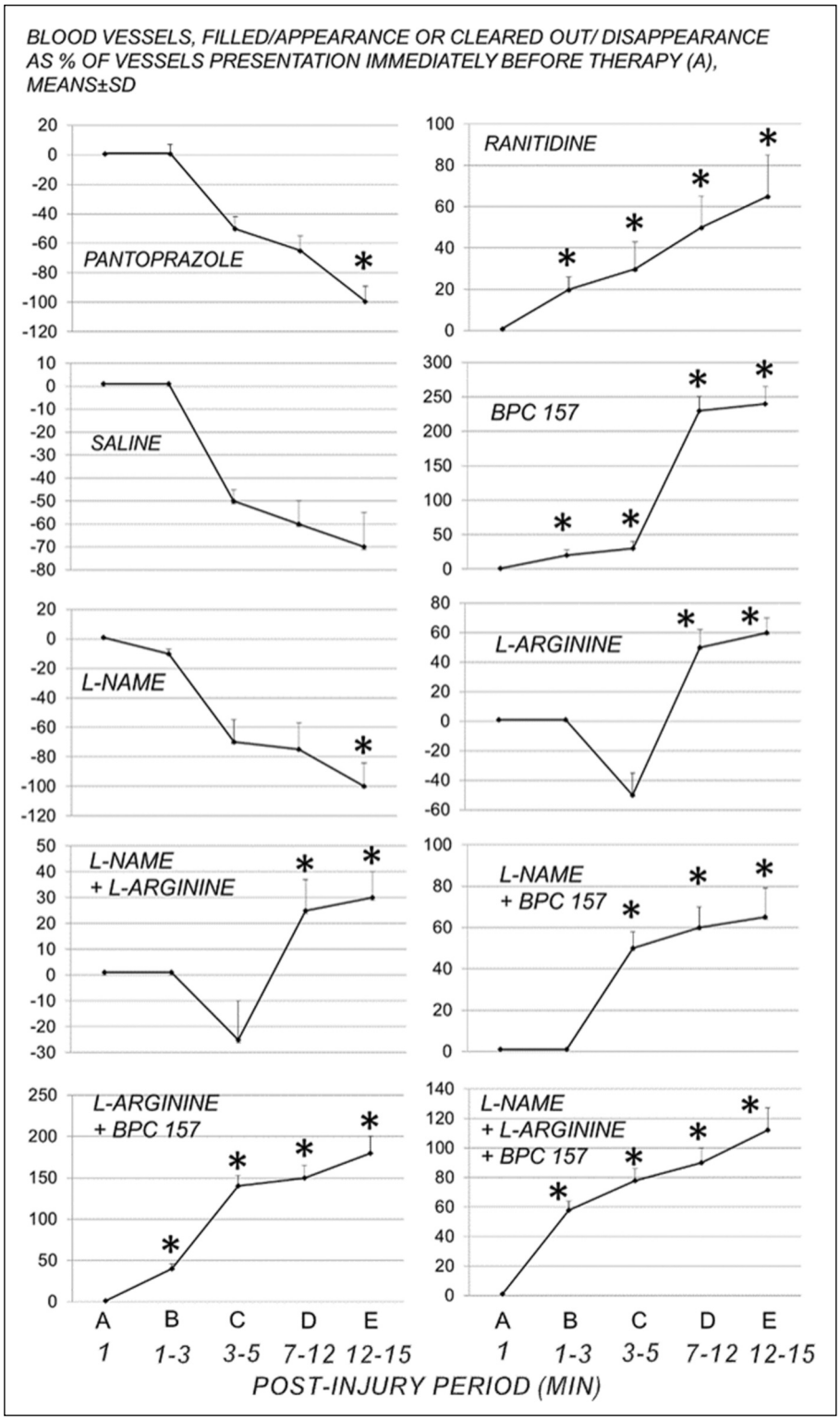

Fig. 1. Blood vessels, filled/appearance or cleared out/ disappearance (assessed with a USB microscope camera Veho discovery VMS-004 deluxe, as % of presentation immediately before therapy (A)) close to the prepyloric defect. 6 rats per each group. (A): after perforation (1 min); (B): during application (2 min); (C): period after application (2 min); (D): next 5 minutes period; (E): period till the end of the observation (15 min). At 1 min post-injury, medication (/kg, 10 ml/2 min bath/rat) at the perforated (5 mm diameter) antral lesion and stomach, includes BPC 157 (10 μg), NOS-blocker L-NAME (5 mg), NOS-substrate L-arginine (100 mg) alone or combined; proton pump inhibitor pantoprazole (10 mg) and H_2-blocker ranitidine (10 mg), saline bath equal volume (controls), and rats were left after abdominal closure undisturbed till the sacrifice, at day 1 or day 7. F ratio = 4.577, $P < 0.05$; *- $P < 0.05$ at least vs. control.

treated rats (*Figs. 1*, *2*, *5* and *6*). This occurs also in the L-arginine + BPC 157-, L-NAME + BPC 157- and L-NAME + L-arginine + BPC 157-rats. Although to the less extent, this improvement occurs also with ranitidine, as well as with L-arginine. Contrarily, pantoprazole and L-NAME had an opposite worsening effect, and more vessels 'disappeared'. Given together, L-NAME and L-arginine (L-NAME + L-arginine) did not counteract each other's effect (the effect of L-arginine prevails). These effects accordingly appeared close to the perforation and in the whole stomach. However, it seems that with pantoprazole, L-NAME (worsening), and L-arginine (improvement) the vascular effect close to the perforation is delayed.

Commonly, upon the perforation, the control rats exhibited huge bleeding (*Fig. 4*). With therapy, the bleeding was lessened in rats treated with BPC 157, L-NAME and ranitidine. Contrarily, the bleeding was markedly prolonged with pantoprazole, and even more with L-arginine. Given together, L-

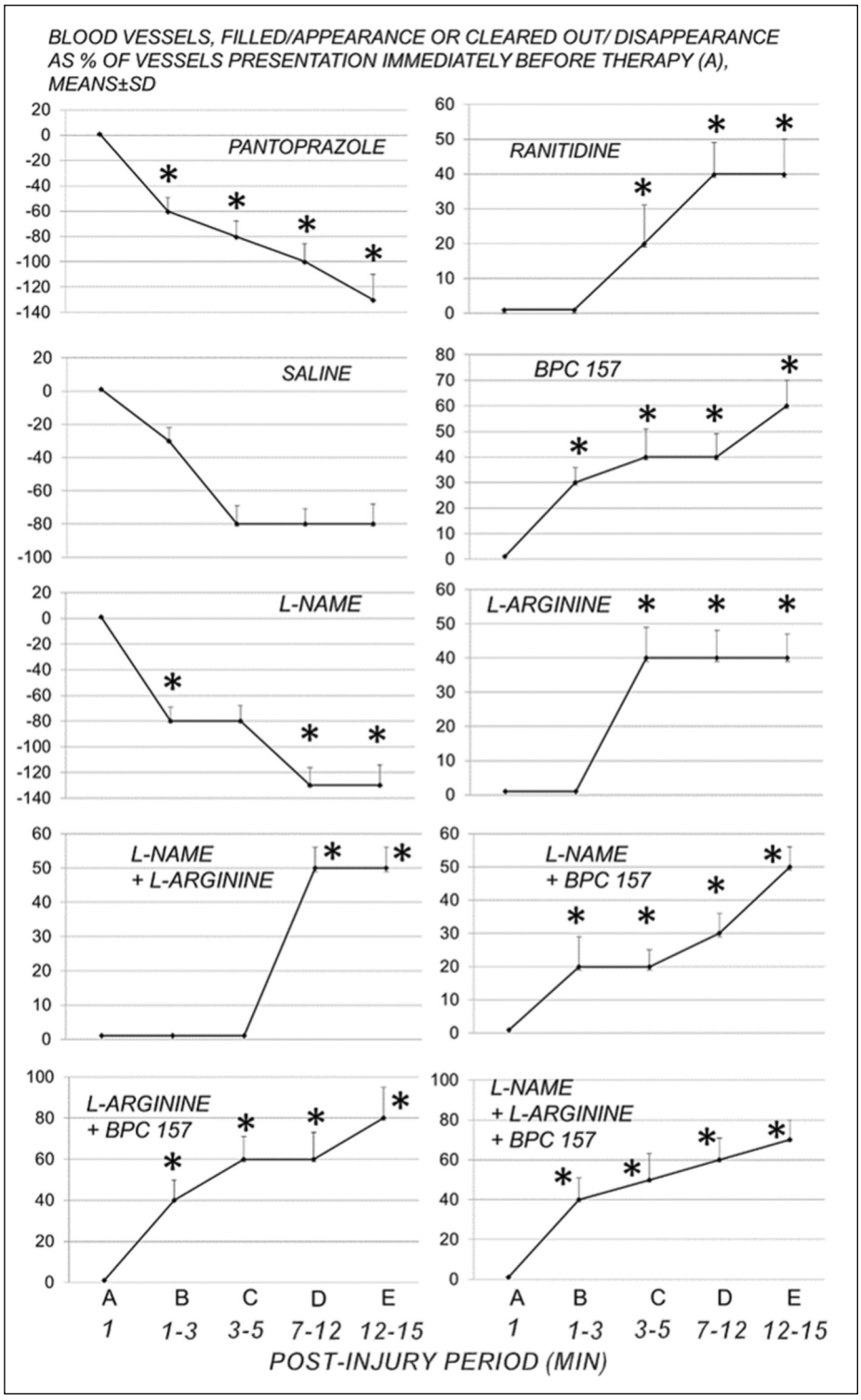

Fig. 2. Blood vessels, fulfilled/appearance or cleared out/ disappearance (assessed with a USB microscope camera Veho discovery VMS-004 deluxe as % of presentation immediately before therapy (A)) in the stomach surface. 6 rats per each group. (A): after perforation (1 min); (B): during application (2 min); (C): period after application (2 min); (D): next 5 min period; (E): period till the end of the observation (15 min). At 1 min post-injury, medication (/kg, 10 ml/2 min bath/rat) at the perforated (5 mm diameter) antral lesion and stomach, includes BPC 157 (10 μg), NOS-blocker L-NAME (5 mg), NOS-substrate L-arginine (100 mg) alone or combined; proton pump inhibitor pantoprazole (10 mg) and H_2-blocker ranitidine (10 mg), saline bath equal volume (controls), and rats were left after abdominal closure undisturbed till the sacrifice, at day 1 or day 7. F ratio = 5.271, $P < 0.05$;*- $P < 0.05$ at least vs. control.

NAME and L-arginine (L-NAME + L-arginine) did counteract each other's effect, thus, the effect similar to the bleeding in the control rats. Rats with perforated stomach exhibited always less bleeding when BPC 157 was given together with L-NAME or L-arginine or with L-NAME and L-arginine (BPC 157 + L-arginine-, BPC 157 + L-NAME- and BPC 157 + L-NAME + L-arginine-rats).

Finally, upon the perforation, it may be the wide defect, huge leaking in the abdominal cavity (*Fig. 3*). Thus, compared to the initial size, the controls exhibited a considerable additional widening of the defect in the early post-injury course. With the post-injury therapy, this spontaneous widening of the defect is markedly lessened after either of pantoprazole, L-NAME and L-arginine as well as after their combination (L-NAME + L-arginine) (L-NAME and L-arginine have a similar effect, and given together (L-NAME + L-arginine) did not counteract each other's effect). With ranitidine, and L-arginine + BPC 157 and L-NAME + BPC 157, this antagonization appears as a more pronounced opposing effect, as the defect that retains its initial size with no further widening in the treated rats. Moreover, after

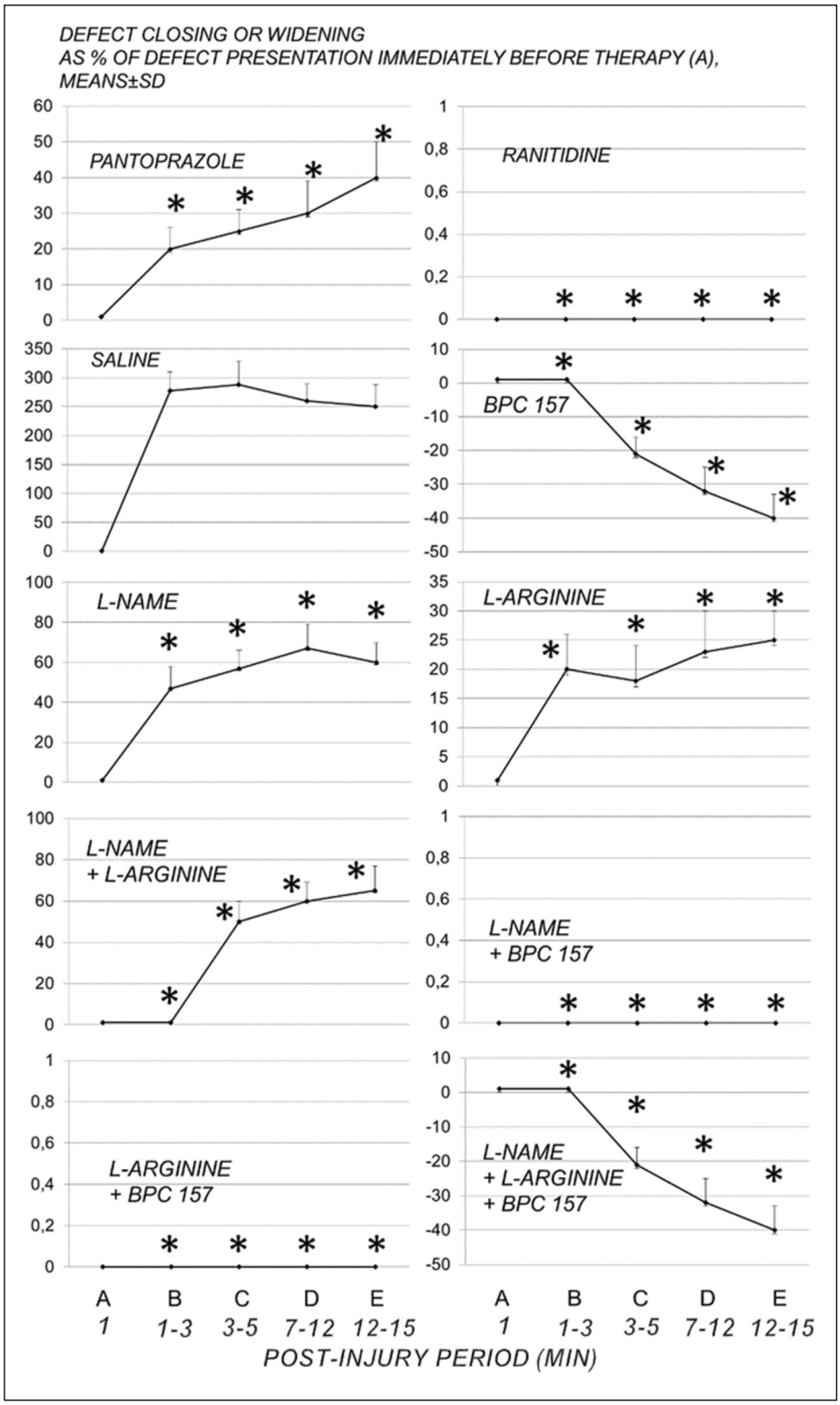

Fig. 3. Defect widening or closing (both as % of presentation immediately before therapy (A)); bleeding time (s); (A): after perforation (1 min); (B): during application (2 min); (C): period after application (2 min); (D): next 5 min period; (E): period till the end of the observation (15 min). 6 rats per each group. At 1 min post-injury, medication (/kg, 10 ml/2 min bath/rat) at the perforated (5 mm diameter) antral lesion and stomach, includes BPC 157 (10 µg), NOS-blocker L-NAME (5 mg), NOS-substrate L-arginine (100 mg) alone or combined; PPI pantoprazole (10 mg) and H_2-blocker ranitidine (10 mg), saline bath equal volume (controls), and rats were left after abdominal closure undisturbed till the sacrifice, at day 1 or day 7. F ratio = 2.832, $P < 0.05$; *- $P < 0.05$ at least vs. control.

BPC 157 medication, this spontaneous widening is reversed to the defect contraction. Interestingly, instead spontaneous widening, the defect contraction appears also in the rats treated with L-NAME + L-arginine + BPC 157.

Furthermore, considering the finding above presented, it is likely indicative that compared to the healthy values, the control rats with the perforated stomach exhibited less NO-values, and increased MDA-values (*Fig. 7*). This effect was completely antagonized by BPC 157 administration, and these rats presented NO- and MDA-values comparable to those in the healthy stomach.

Having ascertained beneficial effect, to a way how BPC 157 may act beneficially in perforated stomach lesion, further support goes with mRNA expression studies (C*ox2*, *VEGFa*, *Nos1*, *Nos 2*, *Nos3*, *Nkap*) done at that very early post-perforation-time (*Fig. 8*). Provided were timely likely specific genes congruence. First elevated was *Nos2* and decreased

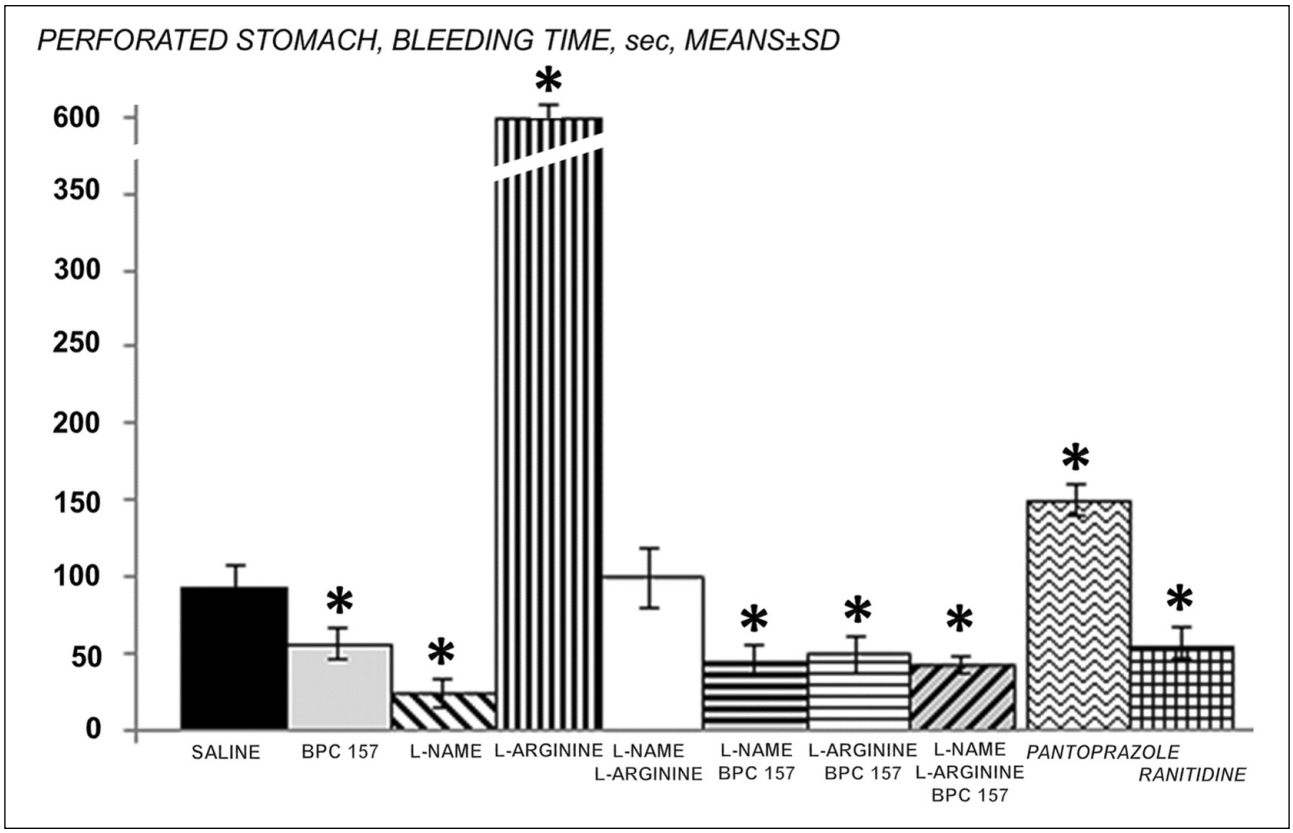

Fig. 4. Bleeding time after perforation. 6 rats per each group. At 1 min post-injury, medication (/kg, 10 ml/2 min bath/rat) at the perforated (5 mm diameter) antral lesion and stomach, includes BPC 157 (10 μg), NOS-blocker L-NAME (5 mg), NOS-substrate L-arginine (100 mg) alone or combined (L-NAME + L-arginine, L-NAME + BPC 157, L-arginine + BPC 157, L-NAME + L-arginine + BPC 157); proton pump inhibitor pantoprazole (10 mg) and H_2-blocker ranitidine (10 mg), saline bath equal volume (controls), and rats were left after abdominal closure undisturbed till the sacrifice, at day 1 or day 7. F ratio = 3.022, $P < 0.05$; *- $P < 0.05$ at least vs. control (S).

VEGFa, then elevated *Cox2*, *Nos1*, *Nos2*, *Nos3*, and then, elevated *Cox2*, *Nos2*, decreased *Nos3* gene expression.

Further course at the day 1 and at the day 7 (*Table 2*) illustrates the advanced defect healing in all rats that received BPC 157 alone or in combinations and less adhesions (note, at the day 7, the healing of the perforated effect is completed at both mucosal and serosal stomach side in the rats that initially received BPC 157 medication). Contrarily, controls appear with the perforated lesion that did not cure, neither at serosal nor at the mucosal side, and considerable adhesions. Indicatively, L-NAME aggravated the perforated lesions (more adhesion severity at day 1 indicates further initial worsening), L-arginine attenuated the lesions (closing however only serosal side), presenting with less adhesions. Given together (L-NAME + L-arginine) oppose each other's effect confirming NO-system related effect. Pantoprazole led to lesions not changed, thereby failed beneficial activity, and no effect on adhesion formation. Ranitidine attenuated the lesions (closing however only serosal side), presenting also less adhesions.

The microscopy findings (*Fig. 9*) closely followed the gross lesion presentations (*Table 2*, *Fig. 5*). For instance, at day 1 post-injury, relatively broad perforations with very intense interstitial edema, diffuse granulocytic inflammation and no tendency of localization were consistently noted in the rats that underwent stomach perforation and received the saline bath. In contrast, in the rats with perforated stomach lesions that received the BPC 157 bath, the edema was much less pronounced; a thick fibrin coating closing the wound towards the peritoneum and adjacent mucosal segments was present; and near adherence was observed (possibly due to reduced edema, muscular layer contraction and fibrin; *Fig. 9*). In the controls at day 7, a wide chronic ulcer with deep-reaching granulation tissue and very rudimentary mucosal overgrowth was observable. In contrast, in the rats that received the BPC 157 bath, mature granulation tissue with a well-developed mucosal overgrowth was present immediately after perforation in the area of the former defect (*Fig. 9*).

DISCUSSION

Taking perforated stomach as additional Robert's prototype lesion made by a direct contact (4), we attempt to rely the rats with the perforated stomach since its very beginning with the cytoprotection concept and therapy resolution (4-8). In this, we evidenced the therapy effects of the stable gastric pentadecapeptide BPC 157, known as cytoprotectant (7), as well as the effects of the given NO-agents (L-NAME, L-arginine and their combinations), and standard agents, pantoprazole and ranitidine as the one bath application to the injured stomach. As precise challenge, this would distinctively affect just the initial post-injury course (BPC 157 > L-arginine, ranitidine > pantoprazole, control > L-NAME). Evidently, the initial event is accordingly decisive for the further healing course and definitive outcome as well.

Within these agents activity limitations (*i.e.* use of the fixed dose), these findings suggest the perforated stomach initially

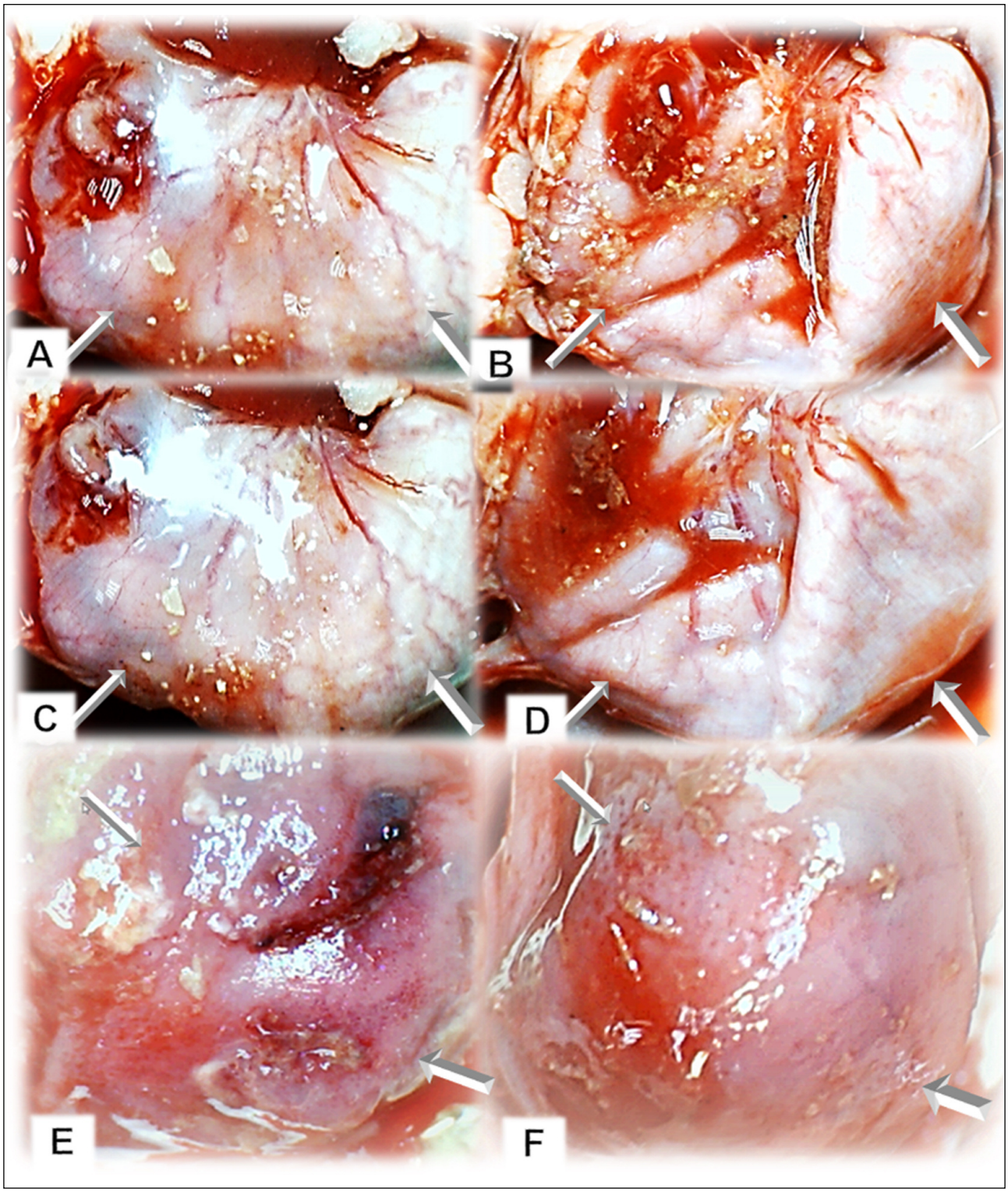

Fig. 5. Perforated stomach presentation, USB microscope camera Veho discovery VMS-004 deluxe. Immediately before therapy (A), (B) (upper) (arrows indicate anterior stomach part with poor vessels presentation). Middle: (C): Weak effect of saline bath (controls) (left, arrows indicate anterior stomach side with still poor vessels presentation). (D): Contrarily, illustrative immediate effect of medication administration, the immediate effect upon BPC 157 bath administration the on blood vessels presentation toward the perforate injury (right, arrows). Lower: (E): Presentation at the day 7 upon stomach opening before sacrifice. In controls, perforate injury (left, upper (arrow) and adjacent injury (arrow). (F): Gross presentation of the area of perforation in BPC 157-rats, 7 days, mucosa presentation without grossly visible defect (arrows).

presented within particular triad immediate presentation (vessels presentation, bleeding presentation, defect presentation) as a particular heretofore-undiscovered pathway in the Robert's stomach cytoprotection concept (4-8) that therapy can modify, accordingly leading distinctively to the corresponding eventual outcome. Thereby, this may be more or less improved, not

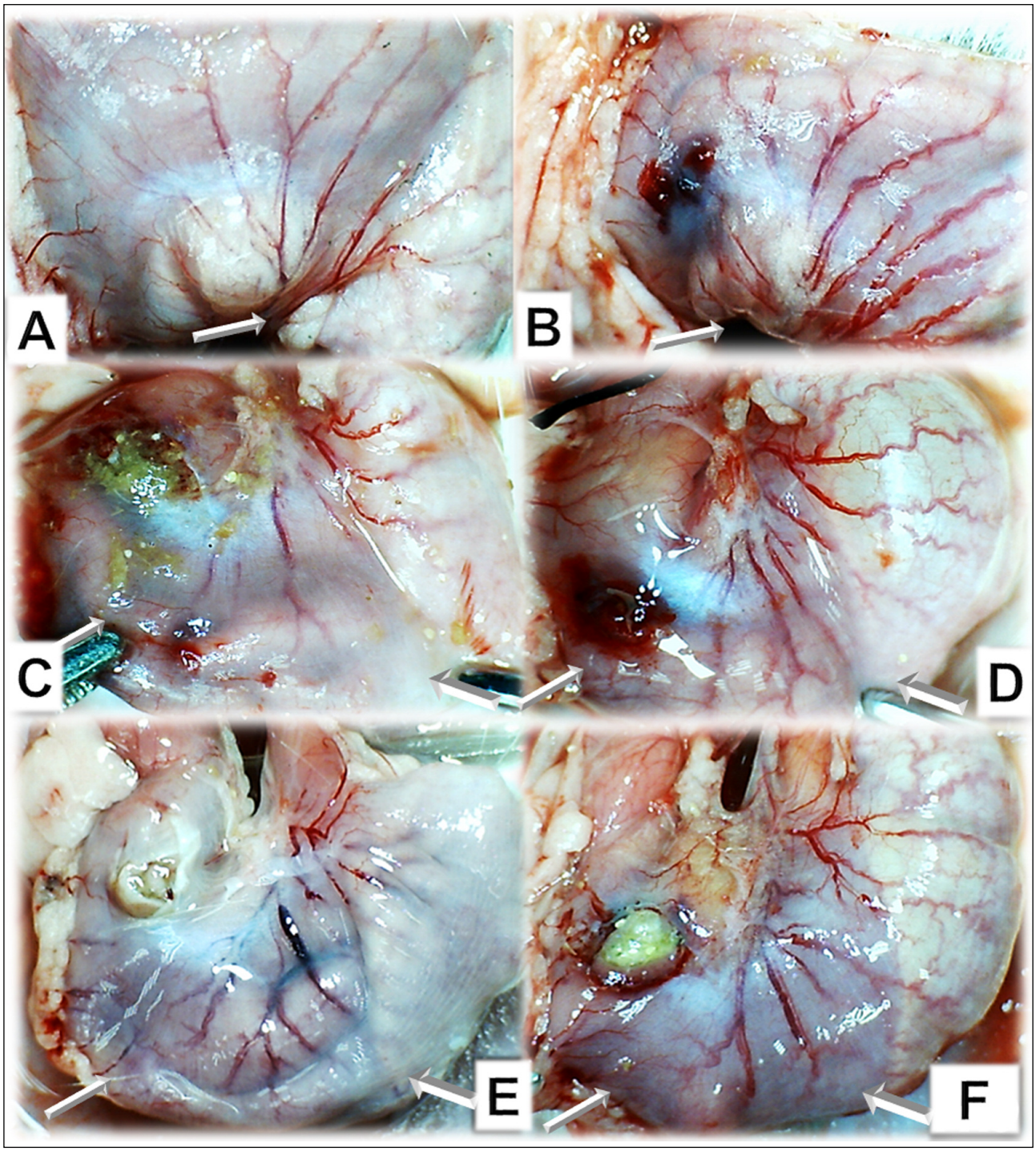

Fig. 6. Perforated stomach, at 10 minutes following medication, USB microscope camera Veho discovery VMS-004 deluxe. Dorsal stomach side presentation (A), (B); ventral side (C), (D), (E), (F) illustrative effect of medication administration (saline bath - left, (A), (C) and BPC 157 bath - right, (B), (D), or (lower) L-NAME bath - left, (E), and L-NAME + BPC 157 bath - right (F). Regular effect of saline bath application on vessels presentation providing poor vessels presentation - left, upper (A), middle (C) (arrows); aggravation by L-NAME bath - left, lower (E) (arrows) versus the immediate effect of BPC 157 bath administration (B), (D), including counteraction of L-NAME-bath-induced aggravation (F), the on blood vessels presentation toward the perforate injury and in damaged stomach (right, arrows).

affected, or worsened defect presentation and adhesion presentation at the day 7. Note, the Robert's/Szabo's original cytoprotective endothelium/epithelium maxim axiomatically works within the minute time (5, 8). Likely, in that rapid pathway, the immediate vessels presentation, bleeding presentation, and defect presentation, while acting in concern, should each specifically respond to the agents' cytoprotective potential.

In the perforated stomach initial triad presentation (failed (vessels failure ('disappearance'/empty), pronounced bleeding, defect spontaneous widening) or recovered (rapidly restored vessels, less bleeding, defect shrinking down) we identified as

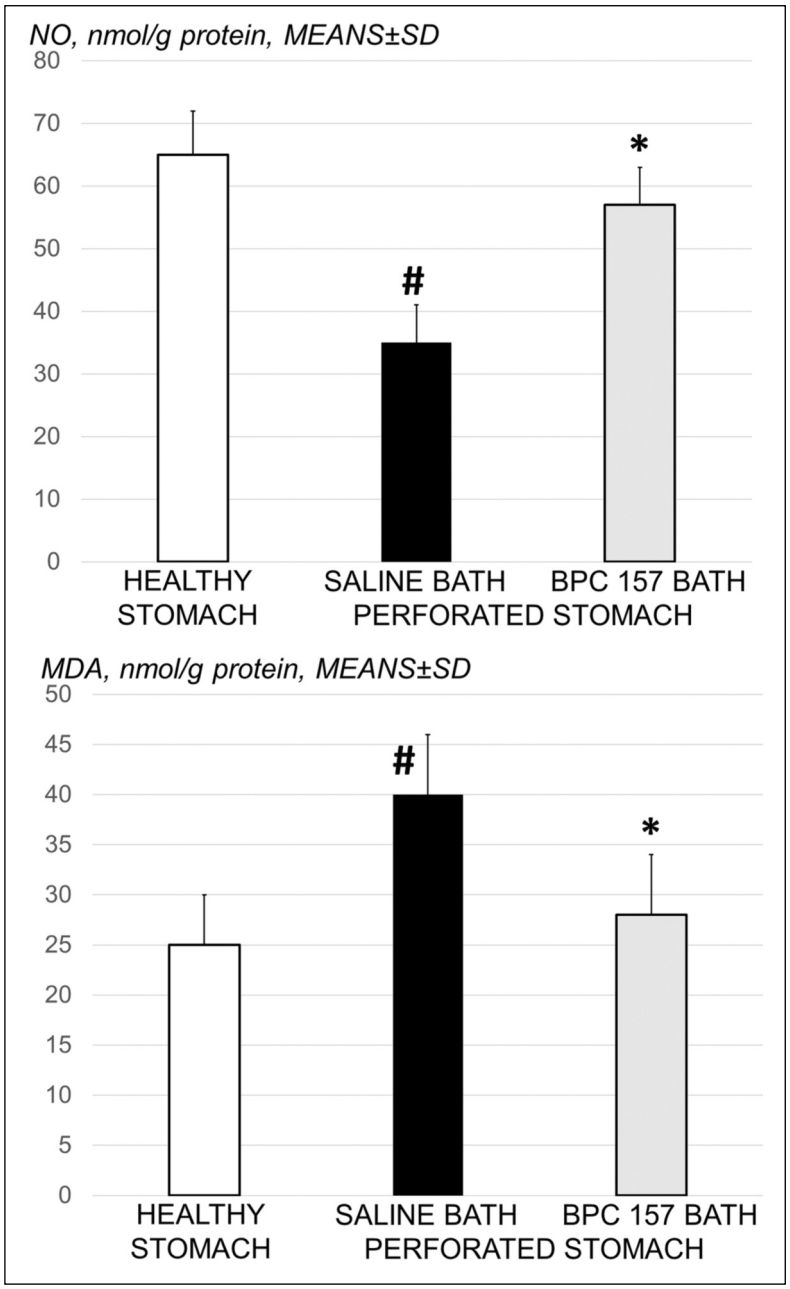

Fig. 7. At the 15 minutes post-injury, we determined nitric oxide (NO) in stomach tissue samples using the Griess reaction (upper), and assessed oxidative stress in tissue samples was by quantifying thiobarbituric acid (TBA) reactivity as malonedialdehide equivalents (MDA). Medication (/kg, 10 ml/2 min bath/rat) at the perforate (5 mm diameter) antral lesion and stomach was BPC 157 (10 µg) or saline bath equal volume (controls). $F = 9.657$, $P < 0.05$ (NO); $F = 6.897$, $P < 0.05$ (MDA);*- $P < 0.05$ at least vs. control, # $P < 0.05$ at least vs. healthy.

two opposed standpoints. The regular negative initial standpoint is reflecting regular failed healing as combined with the eventual poor defect healing and considerable adhesions. For the cytoprotective agents perspective and need that the cytoprotective agents should induce reversal of the damaged tissue to normal structure, interruption of the damaging events (4), the opposite positive initial standpoint was a rapidly operating healing axis as combined with the eventual full defect healing (*i.e.* 'two sides' healing) and considerably less adhesions. BPC 157 regimens fully achieved this essential therapy point. Also, note, at the day 7, the healing of the perforated effect is completed at both mucosal and serosal stomach side in the rats that initially received BPC 157 medication as well as BPC 157 is also known to decrease adhesions formation in other studies (for review see 7, 30, 34, 35, 63-74, 77). The other agents have mixed effects, L-NAME and L-arginine, and combination, and standard agents, pantoprazole and ranitidine vary between these two standpoints. There were the L-NAME vessels worsening, less bleeding, attenuated defect widening, L-arginine vessels improvement, more bleeding, attenuated defect widening. Pantoprazole showed the vessels (worsening), bleeding (prolongation), defect (attenuated widening). Ranitidine exhibited vessels (improvement), bleeding (less bleeding), defect eliminated widening, defect not changed.

The distinctive effects of these agents on the pending vessels, bleeding and defect presentation may reflect their particular contribution to the each of these three initial triad parameters. Likewise, the distinctive effects of these agents may specifically characterized the vessels presentation, bleeding presentation, and defect presentation. Evidently, whether some or all of them appear as NO-system dependent or NO-system not dependent (depending whether L-NAME and L-arginine may or may not antagonize each other effect), responsible to H_2-blockade or proton-pump inhibition, may specify the final defect healing distinctive outcome (eventual lesions and adhesions

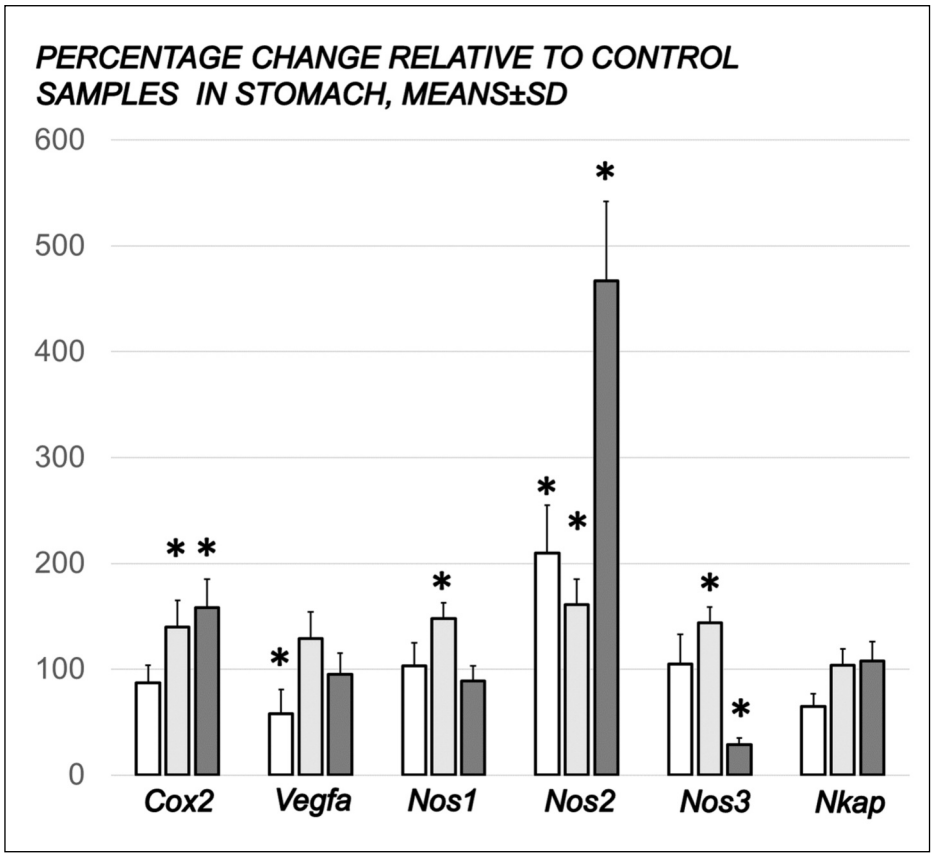

Fig. 8. mRNA expression. Real-time PCR determination of mRNA expression of a set of targeted genes in the stomach tissue samples, at 2 min (white bars), 5 min (light gray bars) and 15 min (dark gray) time interval after medication. Results are expressed as percentage changes (Means ± SD) of BPC 157 10 μg/kg relative to control samples. $P < 0.05$ is marked with *. Results without a * have no biological difference to control samples.

severity attenuation (ranitidine, L-arginine), not influenced (pantoprazole), and worsening (L-NAME)).

Thus, as it had been seen in the recovery of the cecum perforated lesion (23), for the BPC 157 therapy, we may suggest this effect as the upgraded Robert'/Szabo's endothelium/epithelium cytoprotective axiom to the particular blood vessels recovering effect seeable with the perforated stomach recovery. Thereby, it may be that it closely corresponds to the therapy effect in the vessel occlusion and occlusion-syndrome (rapidly activated alternative bypassing pathways to reestablish blood flow continuity) (10-18), and occlusion-like syndrome (major intoxication), maintained intra-abdominal hypertension, grade III and IV) (19-22). To reestablish blood flow, as an innate cytoprotective endothelium effect, the effective upgrading of the bypassing loops of minor vessels to withstand the function of the failed major vessels (10-22) can be essential common principle. Illustrating commonality may be the rat-glaucoma (17), and the rat perforated stomach. In the vessel occlusion and occlusion-syndrome and occlusion-like syndrome studies, there were commonly mitigated lesions in the heart, lung, liver, kidney, and gastrointestinal tract, in particular, and muscle weakness, as well as the lesions in the brain, and the increased intraocular pressure, retinal ischemia in the eye and oxidative stress in tissues (10-22). Specifically, resolved were the pressure disturbances (intracranial (superior sagittal sinus), portal and caval hypertension, aortal hypotension), progressed thrombosis in veins and arteries peripherally and centrally, and thereby stasis commonly resolved (10-22) (note, BPC 157 further improved thrombocytes function without affecting coagulation pathways (10, 23, 33, 47)). Thus, taken together, the rapid vessels 'recruitment', 'running' toward the defect (*i.e*., filled vessels 'appear'; empty, 'disappear') (23) may appear as part of the general defensive effect of BPC 157 therapy application specifically related to the healing of the perforated stomach defect.

Finally, the adequate wound healing involves all four major events (after the loss of vascular integrity, vascular constriction, loose platelet plug, fibrin mesh to insure stability of platelet plug, and dissolution of the clot) subsequently resolved (9). Thus, with the decreased bleeding, vessels recovery and defect shrinking, there is quite consistent evidence in the rats with perforated stomach that the wound healing with stable gastric pentadecapeptide BPC 157 and its innate cytoprotective effect, is evidently accommodated with its particular effectiveness in bleeding disorders (9).

Further characterization of these events went with the NO-system involvement providing its innate role as an endogenously produced vasodilator and its implication in hemostatic mechanisms (30-32), and mentioned BPC 157/NO-system interactions (30). Note, while the fixed dose L-NAME (5 mg/kg) vs. L-arginine (100 mg/kg) vs. BPC 157 (0.01 mg/kg) was taken as limitation, it may be, on other hand, essential for combining the therapy effects in other organs, and distinctive relations demonstration (75-78).

In the early vessels-bleeding-defect relations in the perforated stomach, the relation of the L-NAME (NOS-blockade)/L-arginine (NO-system-over-function)/L-NAME + L-arginine (combined both agents oppose each other response, if NO-system related, NO-system immobilization) is quite complex, providing, however, evidence that the NO-system activities are distinctively presented. The bleeding appeared as typical NO-phenomenon (the opposite effects of L-NAME and L-arginine (more vs. less bleeding) counteract each other effect) (30, 33). Distinctively, vessels presentation (parallel effect of L-NAME and L-arginine) or defect presentation (opposite effect of L-NAME and L-arginine) are thereby different, and most probably, not closely NO-related, likely involving other systems interaction, as L-NAME and L-arginine could not counteract each other effect. At the end (*i.e*. the adhesion formation as

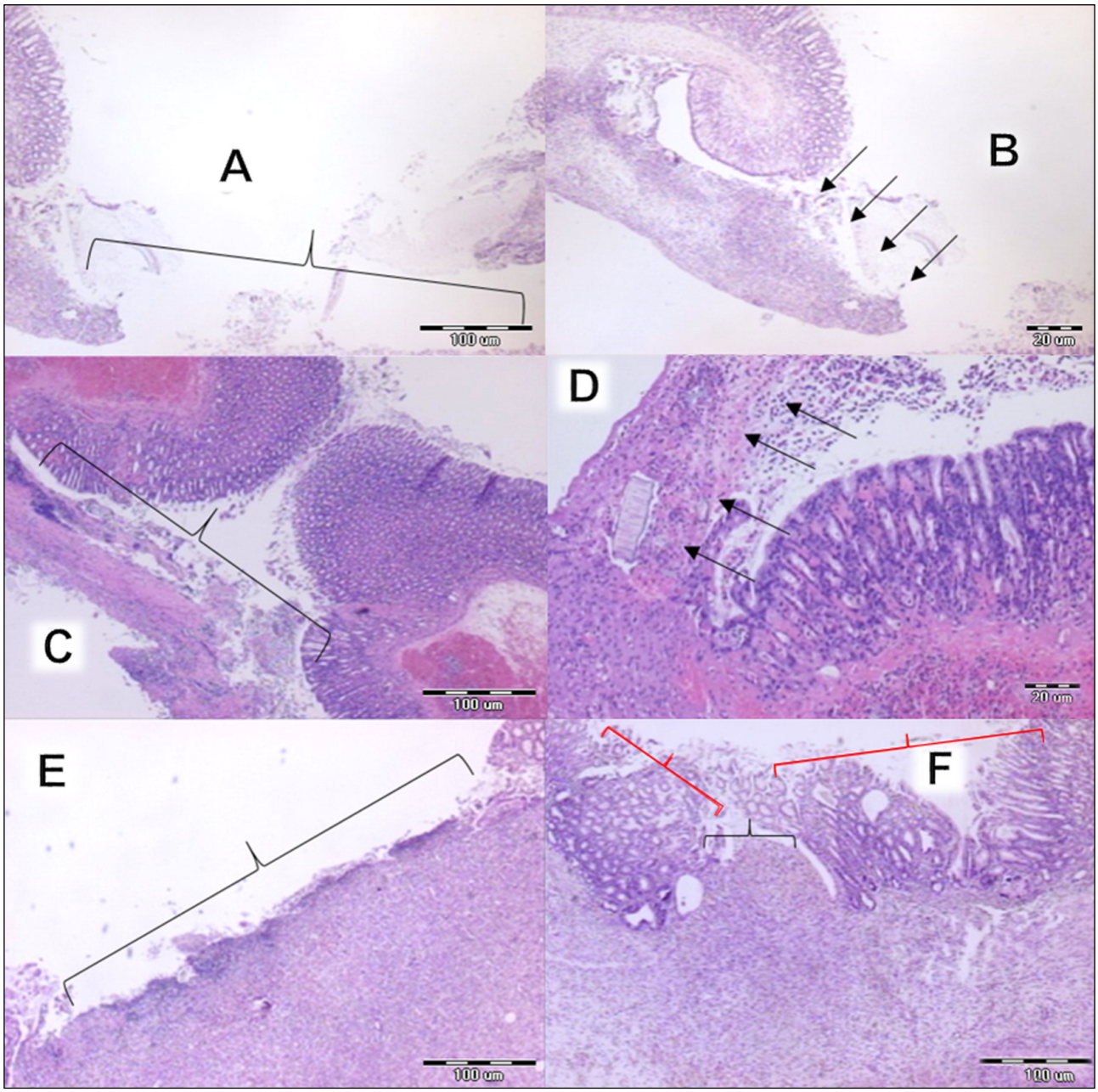

Fig. 9. Stomach perforation microscopy presentation. Upper. Control. Day 1. (A): HE × 4, scale bar 100 µm (left), (B): HE × 10, scale bar 20 µm (right). The perforation is still present (black basic shape). In the surrounding tissue prominent edema and the influx of inflammatory cells can be noted. The surface is covered with fibrin, mucous and cell detritus (arrows). Middle: BPC 157. Day 1. (C): HE × 4, scale bar 100 µm (left), (D): HE × 10, scale bar 20 µm (right). Much lesser edema, exudate with more fibrin and inflammatory cells are forming a 'pseudomembrane' closing the perforation toward peritoneal cavity (black basic shape). First elements of granulation tissue formation apparent in the perforation border zone (arrows). Lower. Day 7. (E): Controls; HE, × 4, scale bar 100 µm (left). A broad area of immature granulation tissue, covered with cellular debris and fibrin is present. Surrounding mucosa shows weak regeneratory changes (black basic shape). (F): BPC 157; HE, × 4, scale bar 100 µm. The area of perforation is permeated by mature granulation tissue (black basic shape) and almost completely covered by regenerating mucosa (red basic shapes).

confirming points, more leaking from the injury, more severe adhesion formation (77)), these responses converge to the final presentation of the defect and adhesions (L-NAME (worsening) vs. L-arginine (attenuation)) as typical NO-phenomenon where the opposite effects of L-NAME and L-arginine (worsening vs. attenuating) counteract each other effect. Evidently, combined triple application may be essential approach (30). It enables closer depiction of the whole NO-system functions (inhibition/(over)-stimulation/immobilization) and demonstrate their distinctive effect depending on the given disturbance and given NO-agent(s) application (30). As an interesting analogy, many distinctive NO-responses simultaneously occurring in the NO-system activation, had been already demonstrated with the counteraction of the resembling 'positive-like' schizophrenia symptoms (*i.e.* 'L-NAME-responsive, L-arginine-responsive', 'L-NAME-non responsive, L-arginine-responsive') (78). However, regardless particularities of the NO-agents effects in the perforated stomach and in other studies (75-78), there is

always particular counteracting potential of BPC 157 application. Illustratively, in the perforated stomach symptoms, as in other studies (75-78), BPC 157 always overwhelmed these L-NAME effects and L-arginine effects (which may not or may counteract each other effect) and in co-administration (L-NAME + L-arginine) restated beneficial effect (L-NAME + L-arginine + BPC 157) (30) and the adverse effects of the lesions that remained are additionally antagonized (30). Thereby, they may be mediated by BPC 157 as well (30).

Note, this L-NAME/L-arginine parallelism occurs with quite distinctive models, and BPC 157 medication counteracted, or, at least, ameliorated myosis, atropine-mydriasis (79), huge magnesium over-dose (80), ischemic/reperfusion colitis (76), duodenal congestion lesions (75), cecum perforation (23). Illustratively, the parallel activity of L-NAME and L-arginine followed the analogous potentiation of acetylcholine (79) or inhibition of dopamine (78) *via* NOS inhibition or the administration of the NOS substrate (78, 79).

Moreover, further particularities are evident also with standard agents, opposite effects of pantoprazole and ranitidine (vessels (worsening, pantoprazole; amelioration, ranitidine), bleeding (prolongation (pantoprazole); attenuation (ranitidine)) and defect (widening (pantoprazole) vs. shrinking (ranitidine)). Thus, we can speculate an intriguing opposition between the proton pump inhibition and H2-receptor blockade in the rat perforated stomach healing.

Having ascertained beneficial effect, further support goes with mRNA expression studies (*Cox2*, *VEGFa*, *Nos1*, *Nos 2*, *Nos3*, *Nkap*) done at that very early post-perforation-time. Provided were timely likely specific genes congruence, firstly elevated *Nos2* and decreased *VEGFa*, then elevated *Cox2*, *Nos1*, *Nos2*, *Nos3*, and then, elevated *Cox2*, *Nos2*, decreased *Nos3* gene expression, as a way how BPC 157 may act beneficially in perforated stomach lesion. Acting through NO-system perceives the high output of NO from iNOS acting in antimicrobial, antiviral, antiparasitic and tumoricidal processes and the cytotoxic effect of NO involved in immunological and tissue-damaging actions (for review see (81)). Likewise is the subsequent acting also through prostaglandins-systems perceiving increased COX-2 as important in various inflammatory and 'induced' settings, and increased prostaglandins, since not stored, synthesized *de novo* from membrane-released arachidonic acid when cells are activated by mechanical trauma (82). These may be also other stimuli (*i.e.*, specific cytokine, growth factor, collagen and adenosine diphosphate (ADP) in platelets, bradykinin and thrombin in endothelium) (82). Both beneficial effects, early (*i.e.*, rapidly recovered blood vessels) and prolonged (*i.e.*, defect healing) by themselves exclude the possible harmful later effect (reperfusion, free radicals formation). Moreover, there is known BPC 157's free radical scavenger effect (10-18, 20, 22, 75, 76, 84-87) and there is clear evidence that the increased MDA- and decreased NO-values in the stomach tissue surrounding defect in the rats with perforated stomach, may be reversed to the normal healthy values in the rats that received BPC 157 medication. Thus, we may suggest an adequate capacity of the oxidant defense system to counteract possible oxidative stress (10-18, 20, 22, 75, 76, 84-87). Possibly, with respect to the underlying disease (which was markedly counteracted in BPC 157 rats), less activity of eNOS (both constitutive isoforms instantly increased along with COX2, and then eNOS decreased) may be an own effect of BPC 157. In particular, this may be substituting NO-effect, additive and/or synergistic effect related to the own activity of BPC 157 administration in the rats with the perforated stomach. Note, a similar combining (BPC 157 beneficial effect/less activity of eNOS-gene) occurred in rats underwent esophagogastric fistulas that were rescued by BPC 157 therapy (53). Likely, the same regulatory contention can be applied for the BPC 157 and VEGFa (initially decreased) and Nkap (seems to be not changed) gene expression, in particular. This may be especially the case, knowing the BPC 157 relation with VEGF (the increased expression and internalization of VEGFR2, the activation of the VEGFR2-Akt-eNOS signaling pathway without need of other known ligands or shear stress (60, 61)), and Nkap-NO relations (NF-κB plays a central role in the regulation of NOS expression) (82). Also, as stabilizer of cellular junction (51), BPC 157 interacts with several molecular pathways (51, 65, 66, 88-90), and that may be along with the evidence that BPC 157 largely interacts with NO-system in various models and species (7, 30, 34, 35, 63-74, 77).

In conclusion, the rats with perforated stomach (as Robert's direct injury by contact (4), as directly made by surgery, hardly heal spontaneously, now presented as particular triad, vascular failure, bleeding, debilitated defect) fairly represent an unresolved cytoprotection issue. Unable endothelium/epithelium maxim, affected distinctive NO-system responses for the vascular failure, bleeding, and debilitated defect (seeable with L-NAME (NOS-blockade)/L-arginine (NO-system-over-function)/L-NAME + L-arginine (NO-system immobilization), and standard agents (in)activity (proton-pump inhibition vs. H_2 receptors blockade)) even overwhelm corresponding clinical emergency. In this, BPC 157 effect (rapidly restored vessels, less bleeding, defect shrinking down, a cluster of the distinctive elements, triad now tightly composed, that all together led to the full healing of the perforated defect and markedly less adhesions) appears as the resolving therapy effect interrupting damaging event that Robert's cytoprotection concept envisaged (4). This may foresee a new quality. This BPC 157's beneficial effect immediately includes the perforated lesion and the whole stomach (note, pantoprazole, L-NAME, and L-arginine have delayed vascular effect close to the perforation). With BPC 157 therapy, this may be the innate upgrading of otherwise unable endothelium/epithelium maxim to a particular blood vessels recovering effect rapidly activated alternative bypassing pathways to reestablish blood flow continuity. In support, this effect was seeable with the counteraction of the severe multiorgan failure (10-22), which appeared as a part of the major vessels occlusion, as well as major intoxication-, maintained intra-abdominal hypertension- and myocardial infarction-induced major vessel failure and Virchow triad circumstances that may be commonly presented (10-22). Finally, increased MDA- and decreased NO-values in the stomach tissue surrounding defect in the rats with perforated stomach, may be reversed to the normal healthy values in the rats that received BPC 157 medication (10-18, 20, 22, 75, 76, 84-87). Along with this, with the possible limitation of results only reflecting mRNA levels, which may not correlate with protein levels (10, 18), mRNA expression studies (*Cox2*, *VEGFa*, *Nos1*, *Nos 2*, *Nos3*, *Nkap*), done at that very early post-perforation-time, indicate a way how BPC 157 may act beneficially in the perforated stomach lesion throughout NO- and prostaglandinds-system. Note, BPC 157 may induce the NO-release of its own (61, 62), even in the condition where L-arginine is not working (62). Furthermore, in addition to being isolated from human gastric juice, BPC 157 was found *in situ* hybridization and immunostaining studies in humans to be largely distributed in tissues (68). Thus, BPC 157, although still clinically infant (*i.e.* used in ulcerative colitis trials) (7, 30, 34, 35, 63-74), may have additional physiologic regulatory roles that may be fully implemented in its profound cytoprotective activity (it does not have a lethal dose (LD1)) (7, 30, 34, 35, 63-74) and may be safely used in purposeful therapy introduction.

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Authors' addresses: Prof. Predrag Sikiric, Department of Pharmacology, University of Zagreb School of Medicine, Salata 11, POB 916, 10000 Zagreb, Croatia.
E-mail: sikiric@mef.hr

Prof. Anita Skrtic, Department of Pharmacology, University of Zagreb School of Medicine, Salata 10, POB 910,10000 Zagreb, Croatia.
E-mail: skrtic.anita@gmail.com