

**GI IUPHAR SECTION
MEETING 2016**

Abstract book

IUPHAR GI Section Symposium on Drug Development and
New Frontiers in Gastrointestinal Diseases

9-11th June 2016
Novigrad, Hotel Maestral, Croatia

Symposium Organizers:

Predrag Sikiric (Croatia)
Duan Chen (Norway)
Josipa Vlainic (Croatia)

Scientific Committee:

Marko Banic (Croatia)
Tomasz Brzozowski (Poland)
Chin Hin Cho (Hong Kong, China)
Marko Duvnjak (Croatia)
Ludmila Filaretova (Russia)
Klara Gyires (Hungary)
Ki Baik Hahm (Korea)
Joshua Ko (Hong Kong, China)
Neven Ljubcic (Croatia)
Gyula Mozsik (Hungary)
Tajana Pavic (Croatia)
Kim Rainsford (UK)
Zarko Rasic (Croatia)
Michael Spedding (IUPHAR General Secretary)
Sven Seiwerth (Croatia)
Miroslav Simunic (Croatia)
Sandor Szabo (USA)
Koji Takeuchi (Japan)
Ante Tonkic (Croatia)
Gabor Varga (Hungary)
Josipa Vlainic (Croatia)

Symposium Secretary:

Domagoj Drmic (Croatia)
Ivan Lerotic (Croatia)
Josipa Vlainic (Croatia) - Chair

Organizer of the Meeting



Supporting institution



SVEUČILIŠTE U ZAGREBU
MEDICINSKI FAKULTET

Technical organizer



MAKS Travel d.o.o.
Nova cesta 60, Zagreb
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Croatian Pharmacological Society

HDPSM HRVATSKO DRUŠTVO ZA PATOLOGIJU
I SUDSKU MEDICINU



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PROGRAM

		IUPHAR GI Section Symposium on Drug Development and New Frontiers in Gastrointestinal Diseases, 9-11 June 2016, Novigrad, Hotel Maestral, Croatia			
Posters Th 14.00 - Sa 14.00					
Th 15.00			Opening		
	Duan Chen (Norway)	Introduction			
	Predrag Sikiric (Croatia)	Introduction			
2016 IUPHAR-GI Section					
Ki Baik Hahm (Korea) ICUR 2018					
HALL A				HALL B	
Hall A	Section chaired by Ki Baik Hahm, Francois Boudreau, Nathalie Perreault, Joshua Ko, Chun Mei Zhao		Hall B	Zarko Rasic, Marko Sever, Leonard Patrlj, Igor Stipancic, Davor Velnic, Mate Skegro, Mario Zovak, Marko Zelic	Fistulas healing and GI-tract healing Sponsored by Croatian Surgical Society
15.30	Ki Baik Hahm (Korea)	8-OHdG as anti-inflammatory, anti-cancer, and anti-metastatic agent through inhibiting ERM signaling in GI diseases	15.30	Marko Sever (Croatia)	Short bowel syndrome and therapeutic possibilities
15.50	Francois Boudreau (Canada)	Role of the nuclear corepressor NCOR1 in gut inflammation and tumor growth control	15.50	Robert Klicek (Croatia)	Ulcerative colitis, complicated with colon-colon anastomosis. Colocutaneous fistulas, acute and chronic, and healing
16.10	Nathalie Perreault (Canada)	Impact of Bmp signaling in the development of gastric reactive mesenchyme and tumor initiation	16.10	Marko Baric (Croatia)	Rectovaginal fistulas healing
			16.20	Tihomir Grgic (Croatia)	Colovesical fistulas healing
16.30	Joshua Ko (Hong Kong, China)	Exploration of the novel anticancer mechanisms of medicinal compounds by regulation of calpain and S100A4 in the treatment of colon cancer.	16.30	Sandra Skorjanec (Croatia)	Gastrocutaneous fistulas healing
			16.40	Sandra Skorjanec (Croatia)	Duodenocutaneous fistulas healing

16.50	Chun Mei Zhao (Norway)	A Potential New Regimen (“Tricyclie”) for Treatment of Gastric Cancer: Targeting Glutamine-dependent WNT/ β -catenin-mTOR Signaling	16.50	Vedran Cesarec (Croatia)	Esophagocutaneous fistulas healing
			17.00	Darko Vukusic (Croatia)	Duodenocolic fistulas healing
	Discussion		17.10	Goran Madjarac (Croatia)	Tracheocutaneous fistulas healing
	Discussion				
	Section chaired by Gaetano Iaquinto, Gabor Varga, Koji Takeuchi, Andre Buret			Zarko Rasic (Croatia), Leonard Patrlj (Croatia)	Fistulas healing and GI-tract healing Sponsored by Croatian Surgical Society
18.00	Antonio De Luca, Giuseppe Mazzarella and Gaetano Iaquinto (Italy)	The role of AIEC in Crohn Disease. Clinical, Immunohistochemical and Pathogenetic Aspects.	18.00	Zeljka Belosic (Croatia)	Novel aspects of lower esophageal and pyloric sphincter function
			18.10	Zeljko Djakovic (Croatia)	Gastroesophageal anastomosis and “sphincter” function
18.20	Gabor Varga (Hungary)	Mesenchymal stem cells as potential tools to treat inflammatory diseases.	18.20	Tomislav Becejac (Croatia)	Cytoprotection and adaptive cytoprotection - a novel view
18.40	Koji Takeuchi (Japan)	Influence of Adrenalectomy on Protective effects of Urocortin I, a Corticotropin-Releasing Factor, Against Indomethacin-Induced Enteropathy in Rats	18.40	Nermin Lojo (Croatia)	NSAIDs and short bowel syndrome
19.00	Andre Buret (Canada)	Enteropathogens disrupt microbiota biofilms to cause post-infectious inflammation.	19.00	Igor Petrovic (Croatia)	Esophagitis and pancreatitis. The relevance of the sphincters function
20.30	Welcome				
2016 IUPHAR GI Section					
	HALL A			HALL B	
Fr	Section chaired by Tomasz Brzozowski, Chi Hin Cho, Klara Gyires, Ludmila Filaretova			Symposium „Update on <i>Helicobacter pylori</i> “ Chaired by: A. Tonkic (Croatia), F. Megraud (France), M. Simunic (Croatia) Sponsored by Croatian Gastroenterological Society	
Fr 9.00	Tomasz Brzozowski	Gaseous mediators in protection of the	9.00	F. Megraud	<i>Helicobacter pylori</i> resistance to antibiotics in Europe

	(Poland)	stomach. Do they interact each other?		(France)	
9.20	Chi Hin Cho (Hong Kong, China)	Vitamin D and its therapeutic targets in the stomach	9.20	M. Katicic (Croatia)	Epidemiology of <i>Helicobacter pylori</i> infection
9.40	Zádori ZS, Fehér Á, Gyires K. (Hungary)	The role of alpha2-adrenoceptors and imidazoline receptors in murine colitis	9.40	A.Tonkic (Croatia)	Treatment regimens for <i>Helicobacter pylori</i>
10.00	Gyires K., Tóth, V., Fehér, Á. Zádori Z. (Hungary)	Endocannabinoids and gastric mucosal defense (oral)	10.00	M. Simunic (Croatia)	Extraintestinal manifestations of <i>Helicobacter pylori</i> infection
10.20	Ludmila Filaretova (Russia)	Rethinking of stress and stress significance in GI tract	10.20	N. Ljubicic (Croatia)	Non ulcer dyspepsia and <i>Helicobacter pylori</i>
	Discussion			Discussion	
2016 IUPHAR GI Section				Symposium chaired by Marko Duvnjak New vistas in hepatology and therapy	
11.00	Susan Hagen (USA)	Tight Junctions in Stomach; Novel Design to Regulate Paracellular Permeability and Prevent Mucosal Injury.	11.00	Marko Duvnjak (Croatia)	Adipokines and Cytokines in the Pathogenesis of NAFLD
11.20	Mozsik,Szabo, Czimmer (Hungary)	Approaches to feedback mechanism system between the membrane-bound ATP-dependent energy systems under different drug actions and different pathological conditions (animal and human observations)	11.20	Lucija Virovic Jukic (Croatia)	Interactions Between Hepatitis C Virus Infection, Insulin Resistance and NAFLD
11.40	Jozsef Czimmer (Hungary)	Central regulation pathways of gastric emptying of caloric food	11.40	Martina Smolic (Croatia)	Structural Based Approach for Inhibition of HCV Replication

12.00	Jack Wood (USA)	Dopaminergic neurotransmission in the ENS	12.00	Marijan Kolovrat (Croatia)	Pringle maneuver and portal hypertension – novel possibilities
Discussion			Discussion		
13.00	Lunch				
2016 IUPHAR GI Section	Chaired by John L. Wallace, Kim Rainsford, Marco Romano, Arunabha Ray		Session <i>Sarcopenia</i> Chaired by Tajana Pavic (Croatia)		
14.00	John L. Wallace (Canada)	H2S-Based Anti-inflammatory Drugs: Lost and Found in Translation	14.00	Tajana Pavic (Croatia)	Skeletal muscle wasting in sarcopenia: molecular pathophysiology
14.20	Kim Rainsford (UK)	Helicobacter pylori and NSAIDs: Two Brethren at Opposite Ends of the Ulcer Spectrum	14.20	Ivan Lerotic (Croatia)	Biomarkers for physical frailty and sarcopenia
14.40	Marco Romano (Italy)	Role of nutraceuticals in treatment and prevention of gastrointestinal disorders.	14.40	Vedran Tomašić (Croatia)	Muscle wasting and cachexia in chronic illness
15.00	Arunabha Ray (India)	The brain-immune axis and stress ulcerogenesis: a pharmacological analysis	15.00	Neven Baršić (Croatia)	Osteosarcopenic obesity: the role of bone, muscle, and fat on health
2016 IUPHAR GI Section	Chaired by Kavita Gulati, Sandor Szabo, Sven Seiwerth, Predrag Sikiric		Chaired by Zarko Rasic, Leonard Patrlj Fistulas healing and GI tract healing Sponsored by Croatian Surgical Society		
16.00	Kavita Gulati (India)	Sexual dimorphism during stress gastric ulceration and its regulation by nitric oxide : an experimental study	16.00	Leonard Patrlj (Croatia)	Anorectal fistulas
16.20	Sandor Szabo (USA)	Ulcerative & inflammatory GI lesions: Prevention and/or treatment?	16.20	Igor Stipancic (Croatia)	Enteroenteral fistulas

16.40	Sven Seiwerth, Lovorka Batelja, Danijela Kolenc (Croatia)	BPC 157 story – pathology view point			
17.00	Predrag Sikiric (Croatia)	BPC 157 story – pharmacology view point			
	Discussion			Discussion	
2016 IUPHAR GI Section	Chaired by Yvette Tache, Ronnie Fass, Oksana Zayachkivska			Chaired by Zarko Rasic, Leonard Patrlj Fistulas healing and GI tract healing Sponsored by Croatian Surgical Society	
18.00	Yvette Tache (USA)	New insight about stress and modulation of visceral pain	18.00	Marijan Knezevic (Croatia)	Superior mesenteric artery and vein obstruction – our insight
18.20	Ronnie Fass (USA)	Non-medical therapeutic modalities for GERD: What's in the toolbox?	18.20	Antonija Duzel (Croatia)	Ischemic colitis – further possibilities
18.40	Ronnie Fass (USA)	How to optimize medical treatment for GERD in 2016	18.40	Kresimir Luetic (Croatia)	Cyclophosphamide application and gastric and duodenal ulcer
			18.50	Tatjana Pavelic Turudic (Croatia)	Cysteamine model – novel application
19.00	Oksana Zayachkivska (Ukraine)	Stress and foregut: novel pharmacological implications.	19.00	Zdenko Bilic (Croatia)	Perforated ulcer
			19.10	Lidija Berkopic (Croatia)	Parietal peritoneum excision and adhesion formation
	Discussion			Discussion	
20.30	Dinner				
2016 IUPHAR-GI-Section					
HALL A			HALL B		
Sa	GI TRACT AND CARDIOLOGY			P. Malfertheiner (Germany); P. Portincasa (Italy) L. Herszeny (Hungary). A. Dorofeyev (Ukraine); G. Nardone (Italy), T. Milosavljević (Serbia), A. Guglietta (Spain); N. Joksimović (Macedonia), B. Tepes (Slovenia), M. Šimunić (Croatia), Ž. Krznarić (Croatia); D. Štimac (Croatia)	
				Protection of gastroduodenal mucosa: from drugs to bacteria Scientific organizers: Adriatic Club for Mucosal Immunology, Croatian Society for Mucosal Immunology (Croatian Medical Association) and Section for Mucosal Immunology, Nutritive Allergy and Intolerance of Croatian Society of Gastroenterology	

9.00	Martina Lovric Bencic, Ivan Barisic, Dijana Balenovic, Sandra Uzun, Dean Strinic, Gordana Zivanovic Posilovic, Mario Udovicic, (Croatia)	Chaired by Martina Lovric Bencic BPC 157 – link between GI-tract and cardiovascular system	9.00		- The influence of PPI use on gastric microbiota
			9.20		- Patients on long-term PPI use for gastroduodenal protection: risks and benefits
	Chaired by Nathalie Vergnolle, Ludmila Filaretova				
9.40	Nathalie Vergnolle (France)	Proteases and Inflammatory Bowel Diseases: what's up Doc??.	9.40		- Gastroduodenal mucosa and H- pylori: what is new?
			9.50		- burning issues in the field
10.00	Yarushkina N., Bagaeva T., Filaretova L. (Russia)	Analgesic effect of Corticotropin-Releasing Factor (CRF): involvement of CRF receptors subtype 1 and 2, opioid receptors and glucocorticoid	10.00		- burning issues in the field
			10.10		- burning issues in the field
	Discussion		10.30		- GI mucosa and xenobiotics in health and disease (20 min)
			10.50		- Nutritive allergy and nutritive intolerance: what is the evidence based difference?
IUPHAR GI Section	Chaired by Sandor Szabo, Peter Konturek, Duan Chen, Predrag Sikiric				
11.00	Sandor Szabo (USA)	Ulcer development and healing: A life-time focus on vascular factors	11.10		- Ecoimmunonutrition and mucosal health (20 min)
11.20	Peter Konturek (Germany)	Modulatory effect of the intestinal microbiota on the brain gut axis	11.30		- burning issues in the field
11.40	Duan Chen (Norway)	Targeting the vagus nerve for obesity control	11.40		- burning issues in the field
			11.50		- burning issues in the field
12.00	Ivana Tlak Gajger and Josipa Vlainic(Croatia)	Appliance of BPC 157 for <i>Nosema</i> spp. invasions control in honeybee colonies (<i>Apis mellifera</i>)			
	Discussion				

13.00	POSTERS REVIEW				
13.40	Duan Chen (Norway)	Concluding			
	Predrag Sikiric (Croatia)				
	Tomasz Brzozowski (Poland)	9th International Symposium on Cell Tissue Injury and Cytoprotection/ Organoprotection			
	Koji Takeuchi (Japan)	IUPHAR 2018			
14.00	Lunch			Lunch	
			15.00		Pharmacological aspects of mucosal protection beyond PPIs
			15.20		Gut microbiome and human health
			15.40		Gut microbiome and probiotics: evidence based story

Joshua Ko

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INVITED LECTURE

Exploration of the novel anticancer mechanisms of medicinal compounds by regulation of calpain and S100A4 in the treatment of colon cancer

Tanshinones have recently been proposed to possess anticancer activity. The present study aimed to investigate the underlying anti-carcinogenic mechanism of cryptotanshinone (CPT). CPT caused an initial upregulation of glucose-regulated protein (GRP)78 in HCT 116 colon cancer cells by eliciting the endoplasmic reticulum (ER) stress response, followed by prolonged apoptosis. For the first time, we discovered that CPT caused rapid and sustained increase in cytosolic calcium in colon cancer cells, which was blocked by pre-treatment of the calcium chelator BAPTA-AM through depletion of the ER calcium store. Besides, we also confirmed that CPT significantly increased calpain activity. A dynamic interaction between GRP78 and calpain under CPT-induced ER stress that resulted in provoked apoptotic activity was unveiled, which could be abolished by calpain inhibitor. GRP78 knockdown caused a substantial increase in the sensitivity of cancer cells to CPT-evoked apoptosis and reduction of cell colony formation, which was confirmed to be p53-dependent by using HT-29 or HCT116 p53^{-/-} cells. The combined anticancer effect of CPT and calpain inhibitor was further exhibited in nude mice xenograft. Depletion of calcium stores in the ER activates store-operated calcium entry (SOCE), causing tumor cell migration and metastasis. S100A4 is a calcium-binding protein that is frequently overexpressed in metastatic malignant neoplasm. We have elucidated the regulation of calcium-mediated S100A4 response in modulating the metastatic capacity of colon cancer by first examining the effect of recombinant (r)S100A4 on the migration of the metastatic colon cancer cells LoVo. rS100A4-mediated cell migration and calcium influx were suppressed by selective SOCE inhibitors SKF96365 and 2-APB. Intrasplenic xenograft of LoVo cells was then performed in nude mice to induce liver metastasis. Tumor growth in the spleen was significantly inhibited by either SKF96365 or 2-APB, with significant attenuation of liver metastasis in animals treated with 2-APB. These findings suggest that inhibition of SOCE would prevent rS100A4-induced calcium influx, which ends up with suppression of the rS100A4-mediated cell migration. Findings in our study exemplify the anticancer potential of CPT by regulation of calpain and calcium homeostasis S100A4-mediated SOCE inhibition. These suggest that CPT could be established as a target-specific chemotherapeutic adjuvant in treating colon cancer.

K.D. Rainsford

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INVITED LECTURE

What is the Place of Stress Conditions in NSAID- and *Helicobacter pylori*-Related Upper Gastro-intestinal Ulcerogenesis?

Current views on the pathogenesis of upper gastro-intestinal (GI) ulcers and bleeding (UGUB) associated with intake of NSAIDs and infection with *Helicobacter pylori* (Hp)¹. In past years “stress” was considered a major factor in UGUB², but interest in the role of stress factors has waned with the prominence of pharmaco-therapeutic studies on NSAIDs and HP, and development of anti-ulcer drugs. The increasing relevance of the gut-brain axis has given understanding of the mediation of stress reactions in the central nervous system (CNS)³. Of importance is the recent recognition of post-traumatic stress disorder (PTSD) particularly in war veterans⁴. Historically, soldiers in combat situations experienced massive GI bleeding and often died from this condition⁴. PTSD has recently patients with rheumatoid arthritis (RA)⁵, leading to the possibility that this stress may contribute to manifestation of rheumatic diseases. In laboratory animal models arthritic disease markedly exacerbates ulcerogenic effects of NSAIDs⁶. The possibility that chronic inflammatory disease may contribute to UGUB in patients who have taken NSAIDs and are infected with Hp has not been fully established. Thus, many conditions are embraced under the global term “stress”. For the rheumatic patient there may be socio-psychologic, PTSD- and disease-related factors contributing to what must be regarded as the multi-factorial basis of GI conditions.

1. Venerito, M., Malfertheiner, P. (2010) *Helicobacter* 15:239-250.
2. Feldman, M. et al. (1986) *Gastroenterology* 91: 1370-1379.
3. Murison, R. (2000) *Encyclopedia of Stress*, 2: 191-196.
4. Dubravčić, D. et al. (1994) *Stress Medicine* 10:57-61.
5. Lee, Y.C. et al. (2016) *Arthritis Care Res.*, 68: 292-298.
6. Rainsford, K.D. (1989) In: Szabo, S., Pfeiffer, C.J., *Ulcer Disease: New Aspects of Pathogenesis and Pharmacology*, pp. 3-13.

Maryana Zvir, Andriyana Beliak, Oksana Zayachkivska.

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INVITED LECTURE

Profiles of saliva crystallization and heart rate variability are related with functional upper gastrointestinal disorders

Introduction. The novel International Classification of Diseases, version 11 (WHO, 2015) contains stress-spectrum pathologies, including gastrointestinal diseases. Over the last decade the wide pathogenesis of functional disorders of upper gastrointestinal part, including laryngo-pharyngeal reflux (LPR). Recently we investigate the result of association of two or more lifestyle risk factors had impact in disbalance of autonomic nervous system (DANS) confirmed by heart rate variability (HRV) and circadian rhythmicity. However relationship between early shift in ANS and lifestyle factors from the clinical point of view of LPR is still unknown. Modern medical students (MS) are a special population group characterized with intensive learning performance, which induced enormous workload, ANS disbalance and wide range of functional gastrointestinal diseases.

Aim: to evaluate relation between DANS, lifestyle risk factors, prevalence of LPR and profiles of saliva crystallization (SC) among MS

Methods: 50 MS were interviewed using questionnaire that included stress perception, physical activity, sleep quality and time duration on IT devices and fGID. Anthropometric data, heart rate variability (HRV) and the SC by dehydration of mix saliva were analyzed.

Results: the mean of body mass index was 21.7; overweight was in 4%. Males tend to have slightly lower muscle mass - 39.2% (normal value (N) above 40%) and higher total fat content - 21.4% (N - up to 20.0%). 68% of individuals were physically inactive in their daily life vs 22% were physically active. The time of using IT devices >6 hrs/day was observed in 63%. The good sleep quality was in 54% vs poor in 46% participants. 67% of MS confirmed high stress level and in 30% was reflected lower parasympathetic activity by HRV. Type I SC was found in 16%; II and III - 70%; IV - 14% of students and it correlated with ANS disbalance and fGID.

Conclusion. Association of two or more lifestyle risk factors had impact on ANS balance. The main risk factors in modern lifestyle are prolonged sitting time, excessive interaction with IT devices, increased sleep disorders and stress perception that lead to circadian dysfunction, which is trigger for ANS disbalance in MS. Further studies are required to assess saliva secretome by “omics” biomarkers of ANS disbalance and fGID.

Key words: stress, autonomic disbalance, circadian rhythm, saliva, secretome

Study was approved by local bioethics committee 15.02.2016 (N2)

Jackie D. Wood

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INVITED LECTURE

DOPAMINERGIC NEUROGENIC MUCOSAL SECRETION IN HUMAN JEJUNUM

Background: Dopamine (DA) is a significant neurotransmitter in the integrative functions of the brain and has equivalent importance in the neurophysiological control of a complex of intestinal secretory and motility functions by the enteric nervous system (ENS). The ENS works also with dopaminergic neurotransmission. The aim of the present study was to examine involvement of DA receptor subtypes in neurogenic mucosal secretory functions.

Methods: Measurement of short-circuit current in Ussing flux chambers was used to assess chloride secretion as a surrogate for mucosal secretion. In human jejunal preparations (preps) obtained from fresh segments of jejunum discarded during Roux-En-Y gastric bypass surgeries.

Results: Mucosal secretion was studied in jejunal preps from 27 patients (18♀, 9♂). The immunoreactivity (IR) for D1, D2, D3, D4 and D5 receptor subtypes was coexpressed with the Anti-hu pan-neuronal marker. Application of DA (1-100µM) to the serosal side of the Ussing chambers evoked a concentration-dependent decrease in basal short circuit current (SCC) with an IC₅₀ of 16.04±0.93µM for 12 preps. Action of dopamine on SCC was mimicked by application of the D1 receptor agonist, A-68930 (1-20µM) and suppressed by the D1 antagonist SCH23390 (20 µM) or phentolamine (10 µM). The D2 receptor antagonist L-741626 (30 µM) did not change the inhibitory action of DA on SCC. Presence of DA and A-68930 significantly suppressed the SCC evoked by electrical field stimulation (EFS). Tetrodotoxin (TTX, 1 µM) in the chambers did not prevent the suppression by DA or A-68930 of SCC. Application of DA or A-68930 in the mucosal compartment of the chambers caused a small reduction in SCC. When applied in the serosal compartment, application of the D2 receptor agonist quinpirole (5-30µM) induced three changes of base SCC i.e. increase: 56.41% (n=22), no change: 28.21% (n=11) and slight decrease: 15.38 % (n=6, P>0.05 for SCC amplitude). The D2 receptor antagonist L-741,626 (10 µM), but not the serotonergic 5-HT₃ receptor antagonist Y-25130(5-HT₃, 20 µM) or the 5-HT₄ receptor GR113808 (5-HT₄, 20 µM), suppressed (-)quinpirole-evoked increases in SCC. TTX (1µM) abolished quinpirole-evoked increases in SCC. In cases where quinpirole-evoked decreases in SCC, the action was reversed by co-application of the alpha₂ adrenergic receptor antagonist, idazoxan (10µM) or phentolamine (10µM) in each of 8 preps. The D4 receptor agonist PD168077, applied in the serosal compartment, evoked increases in SCC in 8 of 13 preps. The D4 antagonist L-741742 (20 µM) suppressed PD168077-evoked increases in SCC. Application of the D3 agonist 7-OH-PIPAT (10-30 µM) did not evoke any changes in SCC in 10 preps.

Conclusion: Dopaminergic signaling in neurogenic mucosal secretion is mainly through D1 and D2 receptor subtypes in human small intestine. Stimulation of the D1 receptor suppresses secretion; whereas, D2 receptor stimulation enhances secretion.

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INVITED LECTURE

8-OHdG as anti-inflammatory, anti-cancer, and anti-metastatic agent through inhibiting ERM signaling in GI diseases

8-hydroxydeoxyguanosine (8-OHdG) traditionally has been acknowledged as a marker of oxidative stress-related mutagenesis, could paradoxically exerted potent anti-inflammatory and anti-oxidant action in various models. In this study, we investigated the chemopreventive effects of 8-OHdG in pancreatic cancer metastasis model. We treated with 8-OHdG to Panc-1, pancreatic cancer cell line, in order to observe the anti-metastasis effects. We performed wound migration assay, invasion assay, zymography, immunoprecipitation assay, confocal microscopy, RT-PCR and western blot analysis. Tail vein in vivo models of metastasis in nude mice were used to assess cancer cell metastasis. 8-OHdG had fabulous efficacy on the metastasis of panc-1 cell which was further confirmed by wound migration assay and invasion assay. 8-OHdG suppressed cell migration by inhibiting ERM phosphorylation via reducing of Rho-GTP activation, thereby decreasing of CD44 expression and interaction between ERM and actin. Moreover, 8-OHdG inhibits epithelial-mesenchymal transition (EMT) through down-regulation of Vimentin and up-regulation of Claudin1 and Zo-1 expression via reducing of ERM and FAK pathway. The expression of various MMPs family was also decreased after 8-OHdG treatment. Notably, 8-OHdG prevented formation of lung metastatic lesions in tail-vein models of metastasis in immunodeficient mice. In conclusion, exogenous 8-OHdG, recognized as the product of oxidative stress, elucidates anti-metastasis actions in carcinogenesis. This inhibitory effect of 8-OHdG on the migration and invasion was mediated by blocking interaction between ERM and actin and inhibiting EMT via the suppression of pERM and CD44.

Andre G. BURET

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INVITED LECTURE

Enteropathogen-induced dysbiosis of human microbiota causes intestinal inflammation: From mechanisms to therapy

A wide range of disorders, intestinal as well as extra-intestinal, have been associated with microbiota dysbiosis, including Inflammatory Bowel Disease (IBD) and post-infectious Irritable Bowel Syndrome. The mechanisms remain obscure. Studies investigating mucosal, rather than fecal, microbiota disruptions are lacking. *Campylobacter jejuni* and the Protozoan parasite *Giardia duodenalis* are two enteropathogens for which post-infectious complications have often been reported. **AIM:** To uncover new mechanisms causing intestinal inflammation upon enteropathogen-induced mucosal microbiota dysbiosis. **METHODS:** These studies investigated the effects of *G. duodenalis* or *C. jejuni* on multispecies microbiota biofilms cultured from healthy human intestinal mucosal biopsies. Microbiota were characterized with new generation sequencing (miSeq; microbial identification, diversity, and clustering) and TRFLP, confocal and scanning electron microscopy, and biochemical analysis for biofilm exopolysaccharide. Biofilm and planktonic bacteria were enumerated with the Calgary Biofilm Device TM. Germ-free mice exposed to microbiota were analyzed for inflammatory responses (LUMINEX, ELISA, histology). Neonatal rats were assessed for post-giardiasis intestinal hypersensitivity. Synchronized *C. elegans* were fed microbiota to assess toxicity. Human enterocyte monolayers exposed to the various microbiota were assessed for apoptosis, tight junctional integrity (ZO-1, occludin), and bacterial translocation. H₂S donors were assessed for their ability to stabilize microbiota in the inflamed gut using a rat model of colitis. **RESULTS:** Acute exposure to *Giardia* or *C. jejuni* disrupts human microbiota biofilms. In neo-natal rats, *Giardia* caused post-infectious intestinal hypersensitivity, long after clearance of the parasite, in the small as well as in the large intestine. Following exposure to *Giardia* or *C. jejuni*, planktonic bacteria are released in greater numbers from the mucosal microbiota biofilms, which are thinner and contain less exopolysaccharide, and bacteria belonging to the class Clostridiales become over-represented. In germ-free mice, post-giardiasis dysbiotic microbiota on their own (ie normal human microbiota after exposure to *Giardia*) significantly increase the numbers and size of lymphocyte aggregates in the intestinal mucosa, and augment concentrations of pro-inflammatory IL-1 β in gut tissues. Microbiota rendered dysbiotic by exposure to *Giardia* are able to induce apoptosis, break down epithelial tight junctions, and translocate in human enterocyte monolayers. These dysbiotic microbiota also induced lethal paralysis in *C. elegans*, at least in part by abnormally colonizing the worm's intestine. Oral administration of H₂S donors stabilized disrupted mucus and microbiota biofilms in the inflamed colon. **CONCLUSION:** *G. duodenalis* or *C. jejuni* modify human intestinal mucosal microbiota biofilms and promote the release of planktonic pathobionts from these biofilms. In turn, these dysbiotic microbiota directly cause intestinal pathology in a live host. We speculate that these may contribute to the development of post-infectious intestinal inflammatory disorders like IBS or flares in patients with IBD, long after the inciting enteropathogen has been eliminated. The therapeutic potential of H₂S-releasing drugs in this context warrants further investigation. **FUNDING:** NSERC, CIHR, AIHS, CCC.

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INVITED LECTURE

Evidence against the role of imidazolin receptors in the regulation of intestinal peristalsis

Introduction: In the last decades three pharmacologically distinct imidazoline receptors (IRs) have been identified (I1-3Rs), which may be promising targets in the treatment of various diseases, including hypertension, diabetes or chronic pain syndromes. Radioligand binding studies revealed the presence of IRs in the gastrointestinal (GI) tract as well, suggesting their role in the modulation of GI functions. In the present study we aimed to analyse the potential role of IRs in the regulation of intestinal peristalsis. **Methods:** The effect of IR ligands on intestinal peristalsis was analysed both in vivo in NMRI and C57BL/6 mice by using the charcoal meal test, and ex vivo by using guinea pig ileum segments. **Results:** Subcutaneous administration of non-selective I1R ligands (i.e. which bind to alpha2-adrenoceptors as well, such as clonidine and rilmenidine) induced dose-related inhibition of GI peristalsis in mice, which was inhibited by pharmacological or genetic blockade of alpha2A-adrenoceptors. The selective I1R and I2R ligands AGN 192403 and 2-BFI failed to produce any inhibition on intestinal transit, and had no remarkable effect on the dose-response curve of clonidine. Similar results were obtained from ex vivo studies. **Conclusions:** Our results indicate that IRs are not involved in the regulation of intestinal motility and provide further evidence for the inhibitory effect of alpha2-adrenoceptors.

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INVITED LECTURE

RETHINKING OF STRESS AND STRESS SIGNIFICANCE IN THE GASTROINTESTINAL TRACT

Society mainly looks upon stress as being a negative phenomenon considering it as a killer number 1 and does not value positive stress influences. None the less, by its nature stress is an adaptive reaction of the body. "Stress is the salt of life. Total elimination of stress would be equivalent to death" (Selye, 1976). The stress reaction coordinates the mobilization of the body's defenses helping it to overcome problems that arise. The current active study of preconditioning phenomenon supports the conclusion that stress can increase adaptive defense's capabilities of the body. If the body is not able to overcome stress influence the effect of stress becomes pathological. We use a new approach to identify the mechanisms of pathological stress influences, based on the study of the mechanisms of transformation of protective physiological effects of stress into pathological ones. This approach provides for the search of ways to prevent the transformation of the protective effects of stress into pathological problems.

Our study focuses on the gastrointestinal tract (GI), which is considered to be one of the most vulnerable targets for pathological influences of stress, and on the hypothalamic-pituitary-adrenocortical (HPA) axis as a key hormonal system for stress. Various manifestations of pathological changes induced by stress in the GI tract are a serious medical problem. However, initial effect of stress on the GI tract is protective. We found that the disturbance of the normal stress reaction by the elimination of the HPA axis's functioning leads to negative effects on the GI tract such as the development and aggravation of gastric ulceration. The results prove that stress plays a leading role in the gastric mucosal integrity maintaining. Preconditioning mild stress, which is everyday event of animal and human life, may attenuate the development and aggravation of gastric injury caused by severe stress. Accordingly our data gastroprotective influence of preconditioning mild stress is mediated by glucocorticoids released in response to mild stress. Corticotropin-releasing factor (CRF) is a central mediator of response to stress. Stress and CRF may alter the brain-gut interactions leading to the development of GI disorders, but CRF as well as stress may also play adaptive gastroprotective role. Our results demonstrate that CRF (peripheral exogenous as well as endogenous one) may protect the gastric mucosa against cold-restraint injury through involvement of glucocorticoids and CRF receptors types 1 and 2. The findings further support the idea about protective influences of stress on the GI tract.

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INVITED LECTURE

Targeting the vagus nerve for obesity control

During the past 5 years of the European FP7 research project entitled “Understanding food-gut-brain mechanisms across the lifespan in the regulation of hunger and satiety for health” (Full4Health; www.full4health.eu/), we have been particularly investigating the role of the vagus nerve in gut-brain signalling.

In January 2015, the U.S. Food and Drug Administration approved “vagal blocking therapy, aka vBloc® therapy” for adults with obesity. vBloc® is a pacemaker-like neuroregulatory device that is implanted laparoscopically into the abdominal wall with a flexible lead placed around the vagus nerve above the stomach, which sends intermittent blocking signals to disrupt the vagal signal. The concept behind the design of vBloc® is well in line with the understanding of the important role of the vagus nerve in the gut-brain axis and in the development/progression of obesity. However, in an initial clinical trial in which we were involved and in a subsequent trial, the clinical end point of $\geq 10\%$ EWL was not achieved and the rate of serious adverse effects was clinically significant. In light of these results, it has been suggested that vBloc® should not be recommended for clinical use in the future.

In animal studies, we found that vBloc® activated vagal signalling to the brainstem and hippocampus but blocked vagal signalling to the stomach, leading to increased satiety, reduced food intake and eventually weight loss (15%), possibly through the same mechanism. Moreover, we found that the effect of BTxA could be prevented by vagotomy, suggesting that it acts via the vagus nerve. Based on newly developed knowledge regarding the role of the vagus nerve in satiety signalling and the mechanisms-of-action of vBloc® and BTxA, we have developed a new treatment, i.e., Endoscopic Injection of Botulinum toxin A for Obesity (EIBO). In a phase IIa clinical trial, we administered EIBO twice within a 6 month interval to 20 adult patients with a BMI 35-44. During the study period, the patients received neither additional medical treatment nor lifestyle counselling. One patient was excluded from the analyses due to taking drugs. After 3, 6, and 9 months, 68-74% of patients showed excess weight loss (expressed as %EWL based on BMI 25), with an average of $10.9 \pm 2.7\%$, $13.2 \pm 3.0\%$, $15.3 \pm 3.7\%$ EWL compared to baseline at each time point, respectively.

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INVITED LECTURE

Mesenchymal stem cells as potential tools to treat inflammatory diseases

Mesenchymal stem cells (MSCs) are multipotent progenitors present in tissues such as bone marrow, dental pulp and periodontal ligament. MSCs have been regarded for long as cellular sources for tissue renewal. But MSCs have potent immunosuppressive properties as well. Although their mechanism of action is not completely understood, it is clear that they can inhibit the proliferation and function of B, T and natural killer cells, modulate the activity of antigen presenting cells and induce regulatory T cells, effects mediated by cell-cell contact and secreted factors. Importantly, the therapeutic efficacy of MSCs may additionally be improved by the gene transfer of immunomodulatory factors.

CNS disorders such as Parkinson's disease, Alzheimer's disease, spinal cord and traumatic brain injuries and neuroinflammatory multiple sclerosis are diverse maladies. These disorders all result in massive tissue destruction combined with various levels of inflammation. Although not in the CNS, inflammatory bowel disease in the gut is also a consequence of destructive tissue processes following uncontrolled inflammation with poorly understood pathomechanisms. Similarly, acute and chronic pancreatitis are inflammatory diseases with no specific treatments. A number of attempts have been made to suppress the inflammatory responses in the above listed disorders, but even if various treatments have been found to be beneficial in animal models, they failed or had only limited effects in daily clinical practice.

Although their location is different, there are many similarities in the immunopathomechanisms of these CNS disorders and the various forms of IBD and pancreatitis. As it is very hard or impossible to cure them by conventional manner, novel therapeutic approaches are needed. These methods may include the application of MSCs, which could be isolated from various tissues including the dental pulp and periodontal ligament. Such cells feature transdifferentiating capabilities for different tissue specific cells to serve as new building blocks for regeneration. But more importantly, they are also potent immunomodulators inhibiting proinflammatory processes and stimulating anti-inflammatory ones.

The present overview compares the immunopatomechanisms of the above mentioned CNS and GI disorders. Additionally, the potential use of mesenchymal stem cells is screened. We envision that such efforts will yield considerable advance in the foreseeable future in treatment options for central and peripheral disorders related to inflammatory degeneration.

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INVITED LECTURE

H₂S-Based Anti-inflammatory Drugs: Lost and Found in Translation

There is a rapidly expanding body of evidence for important roles of hydrogen sulfide in protecting against tissue injury, reducing inflammation, and promoting repair. There is also growing evidence that H₂S can be successfully exploited in drug development. H₂S synthesis and degradation are regulated in circumstances of inflammation and injury so as to promote repair and re-establish homeostasis. Novel H₂S-releasing drugs exhibit enhanced anti-inflammatory and pro-restorative effects, while having reduced adverse effects in many tissues. H₂S is a pleiotropic mediator, having effects on many elements in the inflammatory cascade and promoting the resolution of inflammation and injury. It also contributes significantly to mucosal defence in the gastrointestinal tract, and in host defence against infection. There is strong evidence that novel, H₂S-based therapeutics are safe and effective in animal models, and several are progressing through human trials. A better understanding of the physiological and pathophysiological roles of H₂S continues to be restrained by the lack of simple, reliable methods for measurement of H₂S synthesis, and the paucity of highly selective inhibitors of enzymes that participate in endogenous H₂S synthesis. On the other hand, H₂S donors show promise as therapeutics for several important indications.

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INVITED LECTURE

IMPACT OF BONE MORPHOGENETIC PROTEIN SIGNALING IN THE DEVELOPMENT OF GASTRIC REACTIVE MESENCHYME AND INITIATION OF THE NEOPLASTIC CASCADE

Bone morphogenetic proteins (Bmps) are secreted morphogens involved in gastric cellular functions such as proliferation, differentiation and tumorigenesis. Previous studies in genetically-modified mice models have shown that Bmp disruption in both gastric epithelial and stromal cell compartments leads to the development of gastric tumorigenesis. Studies from our laboratory have furthermore demonstrated that abrogation of epithelial Bmp signaling in the stomach alone was not sufficient to recapitulate the neoplastic features associated with total gastric loss of Bmp signaling. Thus, epithelial Bmp signaling does not appear to be a key player in gastric tumorigenesis initiation. These observations hence suggest a greater role for stromal gastric Bmp signaling in the initiation of gastric neoplasia. In order to identify the impact and specific roles played by mesenchymal Bmp signaling in gastric cell function, homeostasis and tumor initiation, we generated a mouse model with abrogation of Bmp signaling exclusively in the gastric mesenchyme (BmpR1a Δ MES). Herein, we were able to expose an unsuspected role for loss of Bmp signaling in leading normal gastric sub-epithelial mesenchyme to adapt into reactive mesenchyme. An increase in the population of activated-fibroblasts suggesting mesenchymal transdifferentiation along with the presence of inflammatory infiltrates as well as fibrosis was observed in the enlarged mesenchymal compartment of the mutant stomach. In addition, the BmpR1a Δ MES mice exhibited spontaneous gastric benign polyps as early as 90 days postnatal. Mesenchymal disruption of Bmp signaling had a dynamic impact on foveolar hyperplasia, parietal cell atrophy and gastric hypochlorhydria. Further analysis confirmed the presence of both intestinal metaplasia and spasmolytic polypeptide-expressing metaplasia in BmpR1a Δ MES mice stomach. These results support the novel concept that loss of mesenchymal Bmp signaling cascade acts as a trigger in gastric tumorigenesis initiation.

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INVITED LECTURE

A Potential New Regimen (“Tricyclie”) for Treatment of Gastric Cancer: Targeting Glutamine-dependent WNT/ β -catenin-mTOR Signaling

Background/aim: Previously, we showed that the WNT/ β -catenin signaling pathway was upregulated in gastric cancer of humans and mouse models and that denervation by either vagotomy or botulinum toxin type A (Botox) injection suppresses gastric tumorigenesis through inhibition of WNT/ β -catenin signaling pathway in mice. In the present study, we sought to develop a new regimen to target the nerve-mediated glutamine-dependent WNT/ β -catenin-mTOR signaling and the cell proliferation. **Methods:** Thirty-four patients with gastric adenocarcinoma and 139 INS-GAS mice, a genetic mouse model of spontaneous gastric cancer, were included. Gene expression profiling and metabolic profiling in the stomachs were performed using Illumina arrays, GeneGo pathway analysis, and Metabolome analysis. Human gastric cancer cell lines, AGS, KATO III, MKN 45 and MKN 74, were used. The drugs used included: Botox® (cleavage of the SNARE substrate SNAP25), RAD001 (mTOR inhibitor), CPI613 (targeting pyruvate dehydrogenase), 5-fluorouracil (5-FU), platinum-based agents including cisplatin and oxaliplatin, and liposomal doxorubicin (Caelyx, nucleosome destabilization).

Results: In patients, neurotrophin signaling, axonal guidance pathway, WNT signaling, L-glutamate pathways and transport were upregulated in cancer vs. adjacent non-cancerous tissues. In INS-GAS mice, denervation inhibited the glutaminolytic pathway, which included reductions in glutamine, glutamate and glycine. It also inhibited the mTOR pathway. Cancer cells in culture were highly dependent on both glutamine and pyruvate. Screening of agents for growth inhibitory effect revealed the following order of potency: Caelyx > RAD001 > CPI613 > oxaliplatin/cisplatin > 5-FU > Botox. Combination of these agents at IC10-50 resulted in either additive or synergistic effects of > 90% growth inhibition. Reduced glutamine concentration increased cisplatin IC50 by 2-3 folds. INS-GAS mice developed advanced gastric cancer at 10-12 months of age, and were then treated with Botox by gastric injections (once a month) along with various combinations of the drugs (see above) given by i.p. injection for 2 months. Tumor size was significantly reduced by combination therapies, i.e. Botox + RAD001 (67% efficacy), Botox + RAD001 + 5-FU + oxaliplatin (67%), Botox + CPI613 (67%), Botox + CPI613 + 5-FU + oxaliplatin (71%), and Botox + Caelyx + 5-FU + oxaliplatin (80%) compared with chemotherapy only. Importantly, the survival rate was significantly increased in mice treated with Botox + RAD001+ CPI613 + 5-FU + oxaliplatin.

Conclusions: Targeting the glutamine-dependent WNT/ β -catenin-mTOR pathway in combination with chemotherapy represents a promising new therapeutic strategy for gastric cancer. We propose “Tricyclie” regimen as local Botox injection with systemic administrations of RAD001/CPI-613 and 5-FU, platinum-drugs and/or Caelyx.

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INVITED LECTURE

Endocannabinoids and gastric mucosal defense

Introduction: It has been suggested that endocannabinoids (anandamide and 2-arachidonoyl glycerol /2-AG/) and synthetic analogs influence the gastrointestinal motility, gastric acid secretion and inhibit the development of experimental gastric mucosal injury. Moreover, elevation of anandamide and 2-AG levels via inhibition of their degradation by fatty acid amide hydrolase (FAAH) and monoacyl-glycerol lipase (MAGL) respectively, protected the gastric mucosa against NSAID-induced lesions.

Aims: to analyse 1. how inhibitors of the degradation of anandamide and 2-AG affect the mucosal lesions in acid independent, ethanol-ulcer model in the rat, 2. where is the site of gastroprotective action (central/peripheral), 3. the potential mechanism of gastroprotective effect.

Result: 1. While the globally active irreversible inhibitor of FAAH enzyme, URB 597 induced mucosal protective effect given intraperitoneally (i.p.), the peripherally restricted FAAH inhibitor URB 937 (i.p.) failed to influence the mucosal lesions. The irreversible inhibitor of MAGL enzyme, JZL184 given i.p. also exerted gastroprotective effect. 2. Both URB 597 and JZL 184 reduced the mucosal lesions following intracerebroventricular (i.c.v.) administration as well, the effect was reversed by the CB₁ receptor antagonist, AM-251. 3. In gastric mucosa, the ethanol-induced decrease of somatostatin, calcitonin gene-related peptide (CGRP) and partially superoxide dismutase (SOD) was reversed by inhibitors of FAAH, MAGL enzymes (i.c.v.).

Conclusions: Increasing the levels of anandamide and 2-AG induces mucosal protection in the stomach. This effect is likely to be mediated by the activation of central CB₁ receptors and in the periphery by elevation of mucosal CGRP and somatostatin levels. Our study suggests that the endocannabinoid system may play a role in the maintenance of gastric mucosal integrity and gastric mucosal defense.

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INVITED LECTURE

ROLES OF THE NUCLEAR COREPRESSOR NCOR1 IN GUT INFLAMMATION AND TUMOR GROWTH CONTROL

Objective: The nuclear co-repressor NCOR1 is a transcriptional repressor that is recruited to chromatin via physical interactions with nuclear receptors including thyroid hormone receptor, retinoic acid receptors and vitamin D receptors, amongst others. NCOR1 was previously discovered to establish repression checkpoints on broad sets of inflammatory response genes in macrophages. NCOR1 is expressed in intestinal epithelial cells and appears to regulate cell proliferation. The aim of this study was to elucidate the exact functions of NCOR1 in intestinal epithelial and colorectal cancer cells. **Methods:** Two conditional intestinal knockout mouse models (*Ncor1* Δ ID Δ IEC and *Ncor1* Δ Exon11 Δ IEC) were generated to specifically disrupt NCOR1 expression in the intestinal epithelium. Experimental colitis was induced in these mouse models with the use of DSS. Intestinal epithelial cells lines were stimulated with IL-1 β and LPS to inflict an inflammatory response. Gene transcripts expression was measured by gene microarrays and RT-qPCR. NCOR1 expression in colorectal cancer epithelial cell lines was knockdown by RNAi. Cell growth and tumorigenesis properties were measured in soft-agar assays and xenografts into nude mice. Expression of target molecules was monitored by RNA-seq, RT-qPCR and Western experiments. **Results:** NCOR1 expression was increased in intestinal epithelial cells during the inflammatory response. *Ncor1* Δ ID Δ IEC and *Ncor1* Δ Exon11 Δ IEC mice were more sensitive to experimental colitis due to a significant lack of epithelial regeneration throughout the DSS treatments. Gene microarray analyses identified resistin-like beta to be significantly induced in these mice, suggesting a deregulation in the gut microflora. The expression of indoleamine 2,3-dioxygenase, a potent antimicrobial and anti-tumour molecule, was also induced in these mutant mice. When NCOR1 was knockdown in the Caco-2/15 and HT-29 colorectal cancer cell lines, these cells stopped proliferating and became senescent. When soft agar assays were performed, a drastic reduction in colonies formation was noted in HT-29 cells depleted for NCOR1 as compared to controls. Xenografts using CD-1 nude mice and HT-29 depleted cells led to a drastic and significant decrease in tumor growth in NCOR1 depleted cells as compared to controls. RNA-seq analyses identified over 300 mutually modulated genes in both Caco-2/15 and HT-29 NCOR1-depleted cells. We were able to classify these genes in pathways related to inflammatory response, carcinoma, digestive tract cancer, invasion and cellular migration. **Conclusions:** Our results suggest that NCOR1 is an important regulator of CRC cell survival and tumorigenic potential. Because of the current pharmaceutical interest in designing specific NCOR1 targeting molecules, these observations could lead to new therapeutic strategies in neutralizing CRC tumour growth.

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INVITED LECTURE

Sexual dimorphism during stress gastric ulceration and its regulation by nitric oxide: an experimental study

Stress ulcerogenesis is a complex pathophysiology and both central and peripheral factors are involved. Since gender differences are reported for stress responses we experimentally evaluated sex based differences in stress gastric ulcer formation. Cold restraint stress (CRS) is an effective model to induced gastric mucosal damage and the number and severity of gastric lesions were assessed in male and female rats. CRS gastric ulcers were seen in rats of both sexes but such ulceration was found to be more intense in males as compared to females.. The NO mimetic, L-Arginine (100 and 500 mg/kg), dose dependently attenuated CRS-induced gastric ulceroigenesis and decreased ulcer number and severity in male rats. In female rats, L-Arginine also induced a gastric cytoprotective effect during CRS but to a much lesser extent. However, NO synthase inhibition by L-NAME (25 and 50 mg/kg) further aggravated such stress ulcerogenesis in both males and females, with aggravations being more extensive in males. Such stress ulcerogenesis and its modulation by NO-ergic agents were accompanied by lowered levels of NO_x and GSH in brain homogenates and plasma, while MDA levels (lipid peroxidation) were elevated in both male and female rats - the magnitude of these changes being greater in males. Studies in female rats showed that pretreatment with formestane (aromatase inhibitor) but not tamoxifen (estrogen receptor blocker) aggravated CRS-induced gastric ulcerogenesis as compared to vehicle treated and stressed rats. Formestane pretreatment also induced greater suppressions in brain NO_x and GSH and elevations in brain MDA, as compared to vehicle treated CRS rats. These results show that gender based differences exist in stress ulcerogenesis and indicate that estrogen-NO interactions plays a key role in such differences. It is inferred that gastric mucosal integrity during stress is under the regulatory control of physiological factors like sexual dimorphism and and lesser severity of such pathology in females may be due oestrogen-NO interactions.

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INVITED LECTURE

The brain--immune axis and stress ulcerogenesis: a pharmacological analysis

The brain-gut axis plays a crucial role in the regulation of gastrointestinal function. Emotional stressors are known to disrupt the physiological homeostasis and the impact of such an imbalance is also reflected on the gut. Stress-induced gastric ulcer formation is a clinically relevant condition for which treatment strategies are far from satisfactory. Complex mechanisms have been forwarded to explain such stress ulceration and both central and peripheral factors have been implicated. Stress is known to have a deleterious effect on most aspects of immune function and neuro-immune interactions have been reported in stress induced angiogenesis. Anxiety and gastric stress ulcer susceptibility is closely associated and the neural substrates are similar. In view of this, we evaluated the possible involvement of immune mechanisms and CNS-immune interactions during stress-induced gastric ulcer formation in experimental animals by using pharmacological tools. Rats were immunized with SRBC and subsequently exposed to restraint stress (RS) and following this both gastric mucosal integrity and immune responses were assessed. RS induced consistent immune suppression in immunized rats which was evidenced by reduced antibody responses, elevated plasma corticosterone and alterations in plasma cytokines levels. These stress induced immune changes were associated with greater incidence and severity of gastric mucosal lesions as compared to the non-stressed control animals. Long term treatment with dexamethasone, dose dependently aggravated both stress-induced immune suppression and gastric ulcer formation. Further, pretreatment with the opioid antagonist, naltrexone, and dose dependently aggravated both RS-induced immunomodulations and gastric ulcerogenesis. The opioid agonist, morphine, on the other hand, showed attenuating effects on both stress-induced immune and gastric responses. Central depletion of noradrenergic stores (by DSP-4) also resulted in aggravations in both stress induced immune and gastric responses. Similar results were seen when these pharmacological agents were administered centrally (icv). These experimental results are suggestive of the regulatory role of a brain-immune axis during stress ulcerogenesis.

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ORAL PRESENTATION

ANGIOTENSIN II TYPE-2 RECEPTOR INHIBITOR REDUCES SEVERITY OF 2,4-DINITROBENZENE SULFONIC ACID-INDUCED COLITIS IN RAT

Objective: Inflammatory Bowel Diseases (IBD), are severe gastrointestinal (GI) disorders, with unknown aetiology, characterized by a chronic intestinal inflammatory reaction, progressively affecting GI functions, as gut motility. During inflammatory events, modifications in the functionality of some enteric modulators could contribute to the pathological changes of GI motor patterns. Angiotensin II (Ang II), the main effector of the renin-angiotensin system (RAS), has been recently reported as novel regulator of GI motility, acting on the specific receptors (AT1R and AT2R) located on the gut wall. Since recent studies have pointed out an involvement of RAS system in GI inflammation, we explored the RAS functionality and its eventual contribution to colonic dysmotility in a rat model of IBD. **Methods:** Colitis was induced in rats by intrarectal administration of 2,4-Dinitrobenzene sulfonic acid (DNBS). Colonic damage was assessed by disease activity index (DAI), macroscopic and microscopic scores, myeloperoxidase activity (MPO) and TNF- α tissue levels on day 6 after induction of colitis. Effects of Ang II on the contractility of colonic longitudinal muscle strips was recorded isometrically. Effect of 6- day intraperitoneal treatment with PD123319 (3 mg/kg) on amelioration of colitis induced by DNBS was examined in separate group of animals. **Results:** Colonic strips from IBD rats presented, compared to control preparations, an altered spontaneous activity, characterised by lower in amplitude contractions, and reduced sensitivity to carbachol (CCh), a muscarinic cholinergic receptor agonist, and to Isoproterenol (Iso), α α adrenergic receptor agonist. Ang II induced a concentration-dependent muscular contraction in both preparation, which was decreased with a rightward shift of the concentration–response curve in IBD animals. Ang II-evoked contraction was reduced, in both preparations, by Losartan, AT1R antagonist. PD123319, AT2R antagonist, caused a significant enhancement of Ang II response only in inflamed tissues. PD123319 effects were mimicked by L-NNA, nitric oxide synthase inhibitor, and by TTX, neural blocker,. The joint application of PD123319 and L-NNA or PD123319 and TTX had no additive effects. Indeed, in IBD preparations, PD 123319 *per se* ameliorated the mechanical activity increasing both the spontaneous contractions and the sensitivity to CCh and Iso. Daily PD123319 treatment ameliorated the severity of colitis, specially reducing DAI and improving mechanical activity. **Conclusions:** Ang II contracts longitudinal muscle of rat colon via activation of post-junctional AT1R. During inflammation, tonic activation of AT2R, coupled to the nitrenergic signalling, counteract AT1 excitatory effects and would contribute to the reduction of muscle contractility. Pharmacological manipulation of the RAS system seems to improve some IBD symptoms and could provide a future therapeutic strategy for treatment of IBD-associated intestinal dysmotility.

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ORAL PRESENTATION

PERCUTANEOUS ENDOSCOPIC GASTROSTOMY – A SINGLE-CENTER EXPERIENCE

Aim: Percutaneous endoscopic gastrostomy (PEG) is a tool of choice for providing long-term enteral feeding to patients with a variety of chronic and degenerative neuromuscular and malignant diseases which cause swallowing disturbances. The aim of this study is to evaluate our single endoscopic center experience with PEG procedure taking into account demographic data, indications, technical problems and related complications.

Materials and methods: The demographic data, complications and follow-up findings of patients who had undergone PEG between April 2013 and April 2016 were examined prospectively using medical files. We have taken into account the recent and long-term complications, the replacement or removal of the tube were recorded, the patients swallowing function were observed.

Results: One hundred twenty seven percutaneous endoscopic gastrostomy and three hundred percutaneous endoscopic gastrostomy related procedures (143 PEG replacements and 157 regular PEG check outs) were performed in 135 adult patients during a three-year period. The median age of the patients was 59 years (range 17-85 years; 88 male gender). Patients with neurological and malignant diseases (head and neck cancer followed by esophageal and stomach cancer) constituted the majority (64.7% and 26.5% respectively). The PEG placement was successful in 97% of cases (3 patients were referred to surgery). During the follow up period overall rate of complications was 26%. Most common minor complications included local stoma site infection (28%), PEG clogging (26%), and granulations (9%) and were successfully treated conservatively; buried bumper syndrome with skin abscess developed in two patient leading to septic complications and death in one of them. In 12 cases PEG tube was removed when patients have recovered their normal swallowing function.

Conclusion: PEG is safe and effective method for enteric nutrition feeding in adults with swallowing disorders. Owing to the lack of sufficient knowledge and the consequent failure to recognize the problems of patients with secondary dysphagia, PEG is still unsatisfactory and underutilized tool for ensuring artificial enteral feeding in today's routine medical practice in Croatia.

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ORAL PRESENTATION

AN ENDOGENOUS DEFENSIVE CONCEPT, NEW CYTOPROTECTION/ADAPTIVE CYTOPROTECTION: INTRAORAL/INTRAGASTRIC STRONG ALCOHOL IN RAT. BPC 157, L- ARGININE, L- NAME.

OBJECTIVE. We suggest an endogenous defensive concept (96%-ethanol intraorally) that substantially revised Robert's gastric cytoprotection/adaptive cytoprotection (strong alcohol instillation intragastrically).

AIMS. 96%-ethanol intraoral application at the tongue, swallowed; immediate overlapping cytoprotection/adaptive cytoprotection, in unbroken sequence, only the minute stomach lesions versus the Robert's intragastric strong alcohol-huge stomach lesions, and the consequences thereof.

METHODS. We challenged 96%-ethanol: (i) intraorally (1ml/rat at the tongue, swallowed, sacrifice at the 1, 5, 15, 30min; 1, 2 and 24h), the tongue as the first target, then esophagus, stomach, duodenum presentation, hitherto not tested; (ii) intragastrically (1ml/rat, sacrifice at 1h); (iii) intraorally 1ml/rat, and then immediately intragastrically, 1ml/rat, (sacrifice at 1h); assessed lesions area (mm²), lower esophageal (LES) and pyloric sphincter (PS) pressure (cmH₂O). Tested agents (/kg intraperitoneally immediately after alcohol) were: stable gastric pentadecapeptide BPC 157 10µg, 10ng, NOS-blocker L-NAME, 5mg, L-arginine, NOS-substrate, 100mg, applied alone and/or together.

RESULTS. 96%-alcohol induced: (i) intraorally, 1min-24h, widespread, but only minute esophageal, gastric, duodenal lesions with intact mucosa, but markedly fallen sphincter pressures; (ii) intragastrically, the extensive stomach ulcerations, widespread tongue, esophagus, duodenum redness; further fallen sphincter pressures (particularly LES); (iii) intraorally, and then immediately intragastrically, only small stomach lesions, less tongue, esophagus, duodenum redness; the attenuated sphincter pressures drop resembles the attenuated lesions. BPC 157 exhibited additional strong mucosal beneficial effect, at least partially rescued sphincter pressures, L-arginine had slight effect (protection) and L-NAME showed aggravation (and thereby, the NO-system involvement) however not involving change in sphincter pressures.

CONCLUSION. We described a new overlapping cytoprotection/adaptive cytoprotection.

Keywords: BPC 157, alcohol, cytoprotection, adaptive cytoprotection, rats

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ORAL PRESENTATION

BPC 157 FISTULA-HEALING EFFECT CLOSES TRACHEOCUTANEOUS FISTULA IN RATS

AIM: BPC 157 is an original anti-ulcer peptide (GEPPPGKPADDAGLV, M.W. 1419) stable in human gastric juice more than 24 hours, successful in trials for inflammatory bowel disease, phase II, wound treatment, no toxicity or side effects were reported, LD1 was not achieved, effective alone without carrier. BPC 157 also closes gastrocutaneous, colocutaneous and oesophagocutaneous fistulas. Thereby, we investigate whether BPC 157-fistulas healing may be relevant in tracheocutaneous fistula healing.

ANIMALS AND METHODS: Rats were underwent to standard horizontal tracheotomy, trachea opened horizontally, no cannula was inserted and edges of the trachea were sutured on to a skin. BPC 157 (10 µg/kg, 10 ng/kg) was applied (i) in drinking water until the animals were euthanized, or (ii) once a day intraperitoneally (first application 30 min following surgery, last 24 h before euthanization, at 3rd, 5th, 7th postoperative day. Inner and outer part of the fistula were measured.

RESULTS: BPC 157 administered intraperitoneally or perorally accelerated healing of tracheocutaneous fistula and showed functional, macroscopic and microscopic healing improvements.

CONCLUSION: BPC 157 fistula-healing effect may close tracheocutaneous fistula.

Keywords: BPC 157, fistula, healing, rats, tracheotomy

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ORAL PRESENTATION

SPINAL CORD INJURY IN RAT – THERAPEUTIC EFFECT OF PENTADECAPEPTIDE BPC 157

Background. Previously pentadecapeptide BPC 157 exhibited a strong healing effect on brain traumas and peripheral nerve injuries.*

Aim. To verify influence of BPC 157 on rat spinal cord injury.

Materials and methods. Deeply anaesthetized Wistar Albino male rats, 350 g b.w., were subjected to surgery (all experimental procedures were approved by the local Ethics Committee): after laminectomy on level L2-L3 (correspond to sacrocaudal spinal cord) 60 second of compression with neurosurgical piston (60-66 g) on exposed dural sac. One single medication (IP) (saline 5ml/kg; pentadecapeptide BPC 157 200ug/kg; 2 ug/kg) was 10 min post-injury and sacrifice was at 1, 2, 4, 12, 26 and 52 weeks. Tail motor function: (0 autotomy; 1 paralysis; 5 normal) and spasticity (1 normal; 5 maximum) were scored daily. Electrophysiology: with signal filter between 50 Hz to 5 kHz a voluntary muscle activity were recorded 100 mm caudal from rat tail base and average motor unit potential was read off. Histology: samples of spinal cord were taken from lesion site, and tail samples 10 mm distal from base.

Results. Tail motor function. BPC 157 rats have significantly higher motor score in all investigated time. Tail spasticity. BPC 157 rats have significantly less spasticity starting from 4 weeks to the end of experiments. Histology of spinal cord. From 2 weeks to the end of follow up BPC 157 rats have less grey matter edema and better preserved motor neurons also less cyst and axonal necrosis in white matter. Electrophysiology of rat tail muscle. Saline rats have significantly greater giants motor potential that confirm well know process of peripheral motor nerve space compensation caused by decreased number of motoneurons. Histology of rat tail. All treatment groups show increased number of small axons and decreased number of large axons starting from 2 week postinjury.

Conclusions. Given after sacrocaudal spinal cord injury, BPC 157 recovered rat tail function with single treatment. Specifically, BPC 157 decreased secondary injury of spinal cord, and consequently, improved neurologic function recovery.

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ORAL PRESENTATION

Esophagocutaneous fistulas healing

We define the esophagocutaneous fistulas as the NO-system failure due to NO synthase blockade by NOS-blockage by L-NAME, that may be consequently counteracted by L-arginine and gastric pentadecapeptide BPC 157, and a therapeutic benefit gained. And thereby, therapy with a particular anti-ulcer peptide, a safe stable gastric pentadecapeptide BPC 157 GEPPPGKPADDAGLV, MW 1419 (LD1 not achieved) may be appealing since successful in inflammatory bowel disease trials, and counteracts esophagitis, sphincters failure, gastrointestinal ulcer and skin ulcer, gastro- or colo-cutaneous fistulas in rats and may reach esophagocutaneous fistulas healing given per-orally as well as parenterally. Methods: We treated cervical esophagocutaneous fistulas in rats throughout four days (both open skin and esophageal defects, with significant leakage) with BPC 157 (given parenterally and perorally) and L-NAME, and thereby, blunted generation of NO, and L-arginine, NO-substrate, alone or in combination with BPC 157. Results: We evidenced esophagocutaneous fistulas as particular, closely inter-related processes of unhealed skin and esophageal defect, unhealed fistula, normally lethal. Particularly related to the NO-system and therapy-dependent, esophagocutaneous fistulas course was improved speedily and prominently (BPC 157, completely counteracted L-NAME effects (L-NAME +BPC 157 and L-NAME + L-arginine+BPC 157 groups), or more slowly with delay and to less extent (L-arginine) or, alternatively, aggravated, rapidly and prominently (L-NAME). BPC 157 was effective given per-orally or intraperitoneally, in ug- and ng-regimens, both defects start to heal in short period with less fistulas leakage no mortality at day 4, failure of PS and LES pressure restored and practically no esophagitis in any animal during whole experiment.

ORAL PRESENTATION

Gastroesophageal anastomosis healing

Aim. Esophagogastric anastomosis in human surgery is part of the surgical treatment of esophageal cancer and some benign conditions. Anastomotic leak is responsible for one third of all perioperative deaths. We suggest an enhanced healing of esophagogastric anastomosis, NO-system dependent, with stable gastric pentadecapeptide BPC 157 which improved healing of various intestinal anastomosis and which was proposed as a therapy in ulcerative colitis (Curr Med Chem 2012;19(1):126-32; Curr Pharm Des 2011;17(16):1612-32). Also, BPC 157 largely interacts with NO-system (Curr Pharm Des 2014; 20(7):1126-35).

Methods. Throughout 4 days after esophagogastric anastomosis creation, rats received medication (/kg ip once daily: BPC 157 (10 μ g, 10ng), L-NAME (5mg), L-arginine (100mg) alone and/or combined). Daily assessment includes damage in stomach (sum of longest diameters, mm), esophagus (esophagitis, scored 0—5), anastomosis (ml H₂O before leak), pressure in pyloric sphincter and in esophagus at anastomosis (cmH₂O), weight loss (g). The values of 68 – 76 cm H₂O for lower esophageal sphincter, and 68 – 74 cm H₂O for pyloric sphincter, were considered to be normal as determined before.

Results. Esophagogastric anastomosis in controls. As seen at the day 4, we noted progressing stomach damage (8.6 \pm 0.3), severe esophagitis (3/4/5), rapid anastomosis leak (6.5 \pm 1.3), decrease of pressure, severe in pyloric sphincter (27.2 \pm 1.3) as well as less pressure assessed in esophagus at anastomosis (50 \pm 1.3) alongside with prominent weight loss (47.5 \pm 2.8). BPC 157. By contrast, BPC 157 rats treated with 10 μ g have almost no gastric lesions (0.2 \pm 0.1) or esophagitis (0/0/1); anastomosis sustains maximal water volume without leakage (20 \pm 1.0); higher pressure in pyloric sphincter (57.2 \pm 1.6), values in esophagus at anastomosis close to normal values in lower esophageal sphincter, alongside with markedly less weight loss (30.5 \pm 1.9) (Means \pm SD, Min/Med/Max, P<0.05, at least vs. control). Rats treated with BPC 157 smaller regimen (10ng daily dose) exhibited similar therapeutic effect. L-arginine. We noted a beneficial effect comparable to that of BPC 157 regimens. L-NAME. By contrast to BPC 157 and L-arginine beneficial effects, L-NAME rats exhibited all parameters of esophagogastric anastomosis course markedly aggravated. Combinations. L-NAME+L-arginine-rats presented control values; L-arginine+BPC 157-rats presented a beneficial effect but no augmentation of the previous separate effects; L-NAME+BPC 157: BPC 157 completely counteracted L-NAME effects, and maintained its original beneficial effect. L-NAME+L-arginine+BPC 157: BPC 157 presented its original beneficial effect. **Conclusions.** Failed esophagogastric anastomosis healing appears as a NO-system disturbance, and BPC 157 in interaction with the NO-system markedly improves the healing of the esophagogastric anastomosis.

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ORAL PRESENTATION

SPECIFIC AND NON-SPECIFIC ORAL MANIFESTATIONS OF INFLAMMATORY BOWEL DISEASE: A CASE SERIES

Objective: Extraintestinal manifestations of inflammatory bowel disease (IBD) can be presented in the oral cavity. Crohn's disease (CD) can be associated with specific and non-specific oral lesions, while ulcerative colitis (UC) can be associated with non-specific oral lesions, only. We present a case series of three IBD patients with oral lesions.

Case 1: A 28-year-old male patient who has been suffering from CD sought help due to pain and lesions in the mouth. A thickened, inflamed and cobblestone oral mucosa with ulcerations were found in the mouth. The patient was diagnosed with specific oral manifestations of IBD. The mouthwash (0.2% chlorhexidine gluconate solution), topical corticosteroid (0.05% betamethasone ointment), and systemic corticosteroid with proton pump inhibitor were used in treatment. After screening for opportunistic infection and tuberculosis he started with infliximab therapy.

Case 2: A 32-year-old female patient with history of UC complained of severe pain in the mouth, which had occurred after stopping corticosteroid therapy. Clinical exam revealed yellowish, slightly elevated, pustules on the erythematous soft palatal mucosa and dorsal surface of the tongue. The diagnosis of pyostomatitis vegetans, a non-specific oral manifestation of IBD was established. The patient was treated with mouthwash (0.2% chlorhexidine gluconate solution), and topical corticosteroid (0.05% betamethasone ointment). The systemic corticosteroid and azathioprine were started.

Case 3: A 26-year-old male patient with history of CD came to our Clinic due to the sensation of burning in his mouth. He was on adalimumab maintenance therapy, and corticosteroid was added due to the CD worsening. During the clinical exam the atrophic tongue surface and white pseudomembranous lesions on the erythematous hard palatal mucosa were observed. The patient was diagnosed with oral candidiasis – as a complication of IBD treatment. The treatment included the mouthwash (0.2% chlorhexidine gluconate solution) and topical antimicrobial gel (2% miconazole gel). Oral lesions were completely healed upon treatment and the patients are free of oral lesions and feeling well.

Conclusion: Therapy of oral lesions includes treatment of the alterations in the oral cavity according to the aetiology, together with primary intestinal disease, which requires a good cooperation between specialist in oral medicine and gastroenterologist.

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ORAL PRESENTATION

PENTADECAPEPTIDE BPC 157 AFTER 70% LIVER RESECTION IN RATS

Stable gastric pentadecapeptide BPC 157 improves liver function and regeneration after 70% liver resection in rats, as it can rescue otherwise severe liver lesions after overdose of paracetamol, diclofenac, ibuprofen, insulin, CCl₄ or chronic alcohol abuse (for review see, i.e., Curr Pharm Des. 2012). Stable BPC 157 was applied (10 ug, 10 ng) (/kg) either ip once daily or in drinking water (0.16 µg; 0.16 ng/ml/12ml/day) till the sacrifice (at day 4, 14, 21, 28). BPC 157-rats maintained the weight, exhibited better liver regeneration based on better liver mass/body weight ratio (i.e., 14 days: 0.037 +/-0.004 (BPC 157) vs. 0.023+/-0.005 (control); 28 days: 0.053+/-0.007 (BPC 157) vs. 0.03 +/- 0.004 (control)) and larger liver volume in pentadecapeptide BPC 157-rats. Unlike constantly increased AST, ALT, bilirubine levels in controls, BPC 157-rats after a short post-surgery increase, mostly presented values comparable to non-resected animals (conforming functional liver regeneration). Microscopically, controls presented larger areas of liver steatosis unlike BPC 157-rats. Although binuclear cells were sporadically seen in controls, there were less hepatocytes with mitosis compared to all BPC 157 regiments.

Key words: Pentadecapeptide BPC 157, liver resection, rat

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ORAL PRESENTATION

PREVENTION OF MALIGNANT ARRHYTHMIAS IN EXPERIMENTAL RAT MODELS – THE LIFE SAVING EFFECT OF PENTADECAPEPTIDE BPC 157.

Introduction: Malignant cardiac arrhythmias (MCA) lead to death if they persist or do not stop. They are presented as ventricular tachycardia (VT), ventricular fibrillation (VF) or high degree AV block. Disturbances depolarization and/or repolarization can be monitored in the electrocardiogram (ECG) and see the changes in intervals (PQ, QRS, QT).

The causes of MCA are cardiac ischemia, hypoxia, sympathetic stimulation, some drugs, electrolyte imbalances or disturbances in heart conductive system. The principle for experimental models is provocation MCA. Today's antiarrhythmic agents used in the prevention and treatment of irregular heart rhythms. Their proarrhythmogenic effects are the principle for experimental MCA models. Materials and methods: We investigated whether BPC 157 is possible antiarrhythmic drug and we have used different Wistar Rats models like toxic effects of isoprenaline (15 mg/kg TT), barium chloride (10 mg/kg TT), calcium chloride (200-400 mg/kg TT), medigoxin (6 mg/kg TT) and with furosemide-induced hypokalemia (100 mg/kg TT).

The ECG electrodes were placed on the rat limbs and the ECG signal was recorded continuously using ECG monitor of 2090 Medtronic programmer connected to digital oscilloscope LeCroy waverunner LT342 which enabled precise recoding. We analyzed the ECG records on paper A4. All models had preventive, therapeutic and control protocols. BPC 157 was administrated intraperitoneally, intragastrically or intravenously in different doses (pg, ng or µg) before MCA provocation (preventive protocol) or after MCA provocation (therapeutic protocol). Saline was administrated in the control protocol.

Results: In preventive protocols was shorter duration intervals and waves, less reduction in amplitude (R,S), without VT/VF, bradycardia and AV blocks or they were deferred and were shorter. In the therapeutic protocols was achieved termination of VT and AV block, conversion to sinus rhythm and normalization intervals (P,PR,QRS,QT) and amplitudes (P, R, S, T). The control protocols had the high mortality rate

Conclusion: BPC 157 prevents and suppresses arrhythmias in the experimental rats models with MCA and may be interesting antiarrhythmic agent because of the cardioprotective effect.

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ORAL PRESENTATION

Gastric pentadecapeptide BPC 157 and short bowel syndrome in rats

Gastric pentadecapeptide BPC 157, safe as an anti-ulcer peptide in trials for inflammatory bowel disease (PL14736, Pliva) successfully healed the intestinal anastomosis and fistulas in rat. Thereby, we studied for 4 week the rats with escalating short-bowel syndrome and progressive weight loss after small bowel resection from 4th ileal artery cranially of ileocecal valvule to 5 cm underneath pylorus. BPC 157 (/kg) (10 μ g, 10 ng) was given per-orally, in drinking water (12ml/rat/day) or intraperitoneally (once daily, first application 30 min following surgery, last 24 h before sacrifice). Post-operatively, the feature of the increasingly exhausted rats' presentation was the loss of the weight appeared immediately regardless of villus height and crypt depth increased twofold and muscle thickness fourfold within the first week, jejunal and ileal over-dilation, disturbed jejunum/ileum relation. Contrary, the constant weight gain above preoperative values started immediately with BPC 157 therapy, peroral and parenteral, and the villus height, crypt depth and muscle thickness (inner (circular) muscular layer) additionally increased (7,14,21,28 day period). Besides, pentadecapeptide BPC 157 rats showed no different jejunal and ileal diameters, constant jejunum/ileum ratio and an increased anastomosis breaking strength. In conclusion, pentadecapeptide BPC 157 could be helpful to cure short bowel syndrome.

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ORAL PRESENTATION

PENTADECAPEPTIDE BPC 157 REDUCED POSTOPERATIVE ADHESION FORMATION IN RATS

Introduction: Peritoneal adhesions even after minor peritoneum injury are common problems after endoscopy or major surgical procedures. Various agents and methods have been investigated in prevention adhesion formation, such as inhibiting collagen synthesis, but mostly with contradictory or few effect. It is known that formation of intraabdominal adhesions is related to local hypoxia and collagen synthesis. We propose for counterreaction an orally active, stable gastric pentadecapeptide BPC 157 (GEPPPGKPADDAGLV, MW 1419) as anti-ulcer peptide efficient in trials for inflammatory bowel disease (PL-10, PLD-116, PL 14736, Pliva, Croatia) and various wound treatment, no toxicity reported, that diminished adhesions after various intestinal anastomosis in rats. **Materials and methods:** Excision of parietal peritoneum (1x2 cm, 2 cm right from median 3 cm laparotomy) with underlying superficial layer of muscle tissue was performed in rats. BPC 157 (10 µg/kg, 10 ng/kg i.p., 1 mL/rat), or an equivolumen of 0,9% NaCl, L-NAME (5 mg/kg), L-arginine (200 mg/kg) given alone or in combinations with BPC 157 in both concentrations. The medication was applied either immediately after surgery, or once daily through 8 days, last application 24 h before assessment on the 9th postoperative day. Adhesions were scored microscopically and macroscopically (Mazuji's classification).

Results: Rats after surgery exhibited consistent adhesion formation the 9th postoperative day. Macroscopically and microscopically, adhesions were firmly formed between peritoneal lesion and intra-abdominal organs, particularly intestine and uterus, mostly formed of collagen I with a large number of inflammatory cells. Animals treated with L-arginine developed more and firmly adhesions in comparison with application of L-NAME what showed diminish of adhesion formation, what is probably due to collagen synthesis inhibition. Generally, BPC 157 reduced adhesion formation, either 10 µg/kg or 10 ng/kg, given either immediately after surgery or once daily through 8 days, when assessed either microscopically or macroscopically. Adhesions in treated animals, if present, were only filmy, easy in lysis, and they showed mostly collagen III presentation and few inflammatory cells.

Conclusion: In addition to IBD therapy, antagonisation of adhesion formation may provide an additional possibility for this pentadecapeptide therapeutic application.

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ORAL PRESENTATION

The impact of pentadecapeptide bpc-157 on healing of gastric perforation lesions: a comparative study in rat model

Background: BPC 157, a pentadecapeptide derived from human gastric juice, has been demonstrated to promote the healing of different tissues, also including gastrointestinal tract tissues. However, the underlying mechanism has not been fully clarified. **Aim:** The hypothesis of this study was that topic application and intraoral administration of pentadecapeptide BPC 157 has positive healing influence on gastric perforation injury in rat model. **Methods:** Deeply anaesthetized Wistar Albino male rats were undergone to stomach perforation 5mm in diameter on ventral surface of thick-walled glandular part of the stomach. Local bath application on ventral surface (Pentadecapeptide BPC 157 2ug/kg, volume 10ml,) was administered at 5 min post-injury throughout next 5 minutes while controls received simultaneously equal volume of saline. Gross blood vessels presentation (percentage of gastric blood vessel recovery recorded by microcamera and measured by calibrated software) was assessed before and during application and also after administration during next 5 minutes. Then, during recovery period animals received BPC-157 in drinking water (2ug/1ml of water, 12 ml per day) while controls received water (12ml per day). Sacrifice was done on the 1(st), 3(rd) and 7(th) day and perforations lesions diameters were evaluated. **Results:** The results showed that BPC 157 administered by local bath improves gastric blood vessels recovery and also given later orally, subsequently leads to much better healing and closing of gastric perforation lesions. **Conclusion:** There is possibly that the healing of gastric perforating lesions is initially mediated by better blood vessel recovery rate in acute phase after injury in animals that were treated with BPC-157.

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ORAL PRESENTATION

DUODENOCUTANEOUS FISTULA IN RATS AS A MODEL FOR “WOUND HEALING-THERAPY” IN ULCER HEALING: THE EFFECT OF PENTADECAPEPTIDE BPC 157, L-NITRO-ARGININE METHYL ESTER AND L-ARGININE

While very rarely reported, duodenocutanenous fistula research might alter the duodenal ulcer disease background and therapy. Our research focused on rat duodenocutaneous fistulas, therapy, stable gastric pentadecapeptide BPC 157, an anti-ulcer peptide that healed other fistulas, nitric oxide synthase-substrate L-arginine, and nitric oxide synthase- inhibitor L-nitro-arginine methyl ester (L-NAME). The hypothesis was, duodenal ulcer-healing, like the skin ulcer, using the successful BPC 157, with nitric oxide-system involvement, the “wound healing-therapy”, to heal the duodenal ulcer, the fistula-model that recently highlighted gastric and skin ulcer healing. Pressure in the lower esophageal and pyloric sphincters was simultaneously assessed. Duodenocutaneous fistula-rats received BPC 157 (10 µg/kg or 10 ng/kg, intraperitoneally or perorally (in drinking water)), L-NAME (5 mg/kg intraperitoneally), L-arginine (100 mg/kg intraperitoneally) alone and/or together, throughout 21 days. Duodenocutaneous fistula-rats maintained persistent defects, continuous fistula leakage, sphincter failure, mortality rate at 40% until the 4th day, all fully counteracted in all BPC 157-rats. The BPC 157-rats experienced rapidly improved complete presentation (maximal volume instilled already at 7th day). L-NAME further aggravated the duodenocutaneous fistula-course (mortality at 70% until the 4th day); L-arginine was beneficial (no mortality; however, maximal volume instilled not before 21th day). L-NAME-worsening was counteracted to the control level with the L-arginine effect, and vice versa, while BPC 157 annulled the L-NAME effects (L-NAME + L-arginine; L-NAME + BPC 157; L-NAME + L-arginine + BPC 157 brought below the level of the control). It is likely that duodenocutaneous fistulas, duodenal/skin defect simultaneous healing, reinstated sphincter function, are a new nitric oxide-system related phenomenon. In conclusion, resolving the duodenocutanenous fistulas- healing, nitric oxide-system involvement, should illustrate further wound healing therapy to heal duodenal ulcers.

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ORAL PRESENTATION

NSAID-S AND SHORT BOWEL SYNDROME

Aim. We challenge beneficial effect of stable gastric pentadecapeptide BPC 157 (GEPPPGKPADAGLV, M.W. 1419, already successfully tested in patients with inflammatory bowel disease (Curr Pharm Des 2011;17:1612-32)) in massive resection and short bowel (Dig Dis Sci. 2009;54:2070-83), NO-system (J Pharmacol Sci 2008;108:7-17) and diclofenac-overloaded rats (Life Sci 2011;88:535-42). Thus, we focused on the effect of pentadecapeptide BPC 157 in rats with short bowel challenged with high diclofenac dose, and interaction with NO system, NOS-blockade (L-NAME), NOS-substrate, L-arginine.

Methods. After the 80%-small intestine resection, diclofenac 12.5 mg/kg i.p., BPC 157(10 µg, 10 ng/kg, i.p./p.o.), L-arginine (100mg/kg i.p.) and L-NAME (5mg/kg i.p.) alone or combined were given. Control rats received 0.9% NaCl i.p./p.o. Rats were sacrificed after 24h. The assessment included bleeding. Time (BT-s), AST, ALT, bilirubine serum values, anastomosis status and macro/micro examination.

Results. All BPC 157 rats (BPC 157 given alone or combined with L-NAME and/or L-arginine) and L-arginine exhibited shorter BT, decreased bilirubine, decreased gastric, duodenal, jejunum, ileum and rectum lesions while L-NAME rats had longer BT than controls, more increased bilirubine, and more gastric, duodenal, jejunum, ileum and rectum lesions. L-arginine+L-NAME rats had all values similar to control rats. In All BPC 157 treated animals microscopically in liver, significantly increased nucleus number was found compared to control group (40+/-6 (BPC) vrs 12+/-7 (con). Control and L-NAME groups also shown larger liver steatotic areas compared to BPC treated groups (>60% con vs 20-25% BPC).

Conclusion. NO-system dysfunction increased diclofenac lesions. BPC 157 abolished diclofenac toxicity in animals with massive small intestine resection, even in conditions of NOS dysfunction.

Keywords: pentadecapeptide BPC 157, short bowel syndrome, diclofenac

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ORAL PRESENTATION

STABLE GASTRIC PENTADECAPETIDE BPC 157 COUNTERACTS BUPIVACAINE CARDIOTOXICITY, A RAT AND IN VITRO STUDY

The cardioprotective potential of stable gastric pentadecapeptide BPC 157 has already been proven considering chronic heart failure, digitalis arrhythmias and hyperkalemia (in vivo and in vitro). Since bupivacaine realizes its toxic effect through the blockade of K⁺-channels as well, this makes BPC 157 a reasonable therapeutic choice.

The ECG abnormalities observed in rats after bupivacaine toxic dose (100 mg/kg) intraperitoneally (i.p.) were bradycardia, atrioventricular (AV) block, ventricular ectopies, ventricular tachycardia, T-wave elevation and asystolia. All the fatalities developed T-wave elevation, AV-block of the higher degree, respiratory arrest and asystolia. These ECG changes were largely counteracted by the BPC 157 administration (50µg, 10µg, 10ng, 10pg/kg i.p.), either 30 minutes before or 1 minute after bupivacaine toxic dose. When BPC 157 was given later (at the 6th minute), after the establishing of notably prolonged QRS interval (20ms), the fatal outcome was significantly postponed (ANOVA, Student Newman-Keuls, Fisher's, P<0.05). Thereby, BPC 157 both successfully prevents and counteracts bupivacaine cardiotoxic effect, even in the presence of a notable prolongation of the QRS complex as a sign of advanced toxicity. Furthermore, bupivacaine effect on cell's depolarization was explored in vitro, using membrane voltages (Vm) of HEK293. Bupivacaine (1 mM) depolarized cells for 2.13 ± 0.38 mV. In the presence of 1 µM BPC 157, bupivacaine induced depolarizations were inhibited (0.38 ± 0.24 mV). All of these findings along with its, so far demonstrated, very safe profile, suggest that stable gastric pentadecapeptide BPC 157 could be useful as a likely antidote for bupivacaine cardiotoxicity.

Keywords: BPC 157, bupivacaine, cardiotoxicity, rats, HEK293 cell depolarization

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ORAL PRESENTATION

APPLIANCE OF BPC157 FOR *Nosema ceranae* INVASION CONTROL IN HONEYBEE COLONIES (*Apis mellifera*)

Honeybees (*Apis mellifera*) are the predominant and most economically important group of pollinators. Honeybee colony declines and losses, which are the result of a multiple stressors impacting independently or synergistically, have caused much concern worldwide. Nosemosis type C caused by the microsporidium *Nosema ceranae* adversely affect honeybee health and can result in the complete colony collapse. Because antibiotics are banded for use in beekeeping of European Union, and there isn't any registered veterinary medical product for nosemosis treatment, one of the biggest challenges put in front of the scientists are further studies of the potential treatment or beekeeping techniques which are urgently required to combat the rapid spread of this dangerous and emerging disease. As BPC157 in many ways may improve the gastrointestinal tract therapy in mammals, in the present field study the effect of repeated applications of this pentadacapeptide as food supplement have been tested to control *N. ceranae* invasions in honeybee colonies.

Experiment was conducted during July and August in 2014 on apiary where naturally infected honeybee colonies were divided in experimental (treated with 0.25 L sugar syrup supplemented with 0.1 µg/mL BPC157, per day) and control group (received 0.25 L pure sugar syrup, per day) during 30 consecutive days. The foragers were collected on 1, 10, 20 and 30 day of initial of feeding. The spore concentration was determined using Bürker-Türk haemocytometer and *Nosema* species by multiplex PCR.

All analysed composite honeybee samples were positive for *N. ceranae* infection. Field study demonstrated that the disease was not completely cured, but BPC157 administrated via sugar syrup as food supplement significantly reduced the development, and consequently, the number of *N. ceranae* spores in midgut of honeybees originated from experimental group (40.3% on 20 day and 68.1% on 30 day as compared to the initial sampling before feeding).

These results demonstrate the efficacy of BPC157 in reducing spores found in the honeybee midgut over a short period of time and suggest that it may limit the mortality rate in bees and be beneficial in nosemosis type C treatment. Further field and laboratory tests are needed to provide stronger evidence on the potential of this supplement. This was the first appliance of BPC157 in managed colonies of beneficial social insects.

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ORAL PRESENTATION

BLEEDING PEPTIC ULCER: EPIDEMIOLOGY, RISK FACTORS AND OUTCOME

AIMS: The aim of this study was to determine the epidemiology, risk factors and outcomes in peptic ulcer bleeding (PUB).

METHODS: A total of 2643 patients referred to the emergency department of «Sestre Milosrdnice» University Hospital Center due to acute upper gastrointestinal bleeding (UGIB) in the period from January 2008 till December 2013 were screened; 1012 of them diagnosed with PUB during urgent upper GI endoscopy (performed within 24 hours of admission) were enrolled in this prospective study.

RESULTS: The cumulative incidence of UGIB in the 6-year period was 123/100000. Two-fifths of the patients had PUB, of which 496 (49%) patients had a bleeding gastric ulcer, 476 (47%) patients had a bleeding duodenal ulcer, 24 (2.4%) patients had both bleeding gastric and duodenal ulcers, and 16 (1.6%) patients had a bleeding ulcer on a gastroenteric anastomosis. PUB was more common in men. Average patient age was 65.3 years. Twenty-eight percent of patients with PUB had been taking non-steroidal anti-inflammatory drugs, 20,3% acetylsalicylic acid, 2,6% antiaggregation medication and 5,8% anticoagulant therapy. Nine percent of patients with PUB were taking PPIs (proton-pump inhibitors) and 10.3% of them were taking histamine-2 (H2) antagonists as protective acid-suppressive therapy. H. Pylori infection was diagnosed in 425 (42%) patients. Red blood cell transfusion was administered in half of the patients with a median of 2,2 units. Rebleeding occurred in 95 (9.4%) patients. Thirty-days morality was 5.2%.

CONCLUSION: PUB is the dominant cause of bleeding from the upper GI tract. It is associated with the use of agents that attenuate the cytoprotective function of the gastrointestinal mucosa, and with H. Pylori infection. Significant rebleeding and mortality rates are major concerns.

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ORAL PRESENTATION

DIAGNOSTIC CHALLENGE OF A PATIENT WITH CHYLOUS ASCITES – A CASE REPORT

Introduction: This case report presents a diagnostic workup for patient with chylous ascites. **Methods and materials:** 71-year old patient who was hospitalized on June 2014 due to chest pain and dyspnea, as a result of massive pleural effusions. Pleural fluid analysis did not show malignancy. Later on patient felt abdominal discomfort and evaluation of gastrointestinal tract was made. Upper endoscopy showed hiatal hernia, H. pylori negative gastric mucosa erosions and bulbitis. Colonoscopy found small sessile polyp in sigmoid colon, which was removed (pathohistological analysis: tubular adenoma with grade I dysplasia). On January 2015 patient was hospitalized again, this time due to ascites and extensive diagnostic evaluation was made including gastrointestinal, pulmonary, cardiac, immunological and hormonal tests. Routine laboratory findings, thyroid hormones and tissue transglutaminase antibody were normal, while tumor marker CA125 was elevated (175 kIU/L). Abdominal fluid analysis was made several times and showed milky-appearing ascites (cytology: mesotel cells; biochemical characteristics: chylous ascites–elevated triglycerides). Antibiotic treatment was carried out due to elevated ascetic leukocytes count. Deep duodenoscopy with duodenal biopsies was done, and patohistological analysis showed intestinal lymphangiectasia. Imaging methods (ultrasound and computed tomography) showed smaller reflective liver, while Doppler ultrasound excluded a portal hypertension signs. Other findings (Quantiferon test, anti-HIV, angiotensin-converting enzyme, 5-hydroxyindoleacetic acid urine test, metanephrines, normetanephrine in 24h urine) was normal, while chromogranin A was slightly elevated (144 µg). Additionally, positron emission tomography and Technetium-99m nanocolloid lymphoscintigraphy was normal. During the next few months patient felt a general weakness and collected ascites was evacuated for few times. **Results:** Finally on May 2015 explorative laparotomy was done. Abdominal cavity was filled with chylous ascites which was mostly aspirated and liver and peritoneal biopsies were done. Patohistologic analysis showed no elements of liver cirrhosis, with elements of peritoneal fiber tissue and striated skeletal muscle tissue. Over next few months patient was followed with diuretic therapy and recommended diet (low-fat diet with medium-chain triglycerides supplementation): he felt better with collection of smaller amount of ascites. **Conclusion:** We presented a case report of patient with massive chylous ascites which required extensive diagnostic workup to exclude different causes (malignancies, cirrhosis, lymphatic abnormalities, inflammatory conditions, miscellaneous disorders, congenital, infective, postoperative and traumatic causes). It is very important to find etiology of ascites due to different treatment options. Etiology of ascites in this patient is not clear yet and further reevaluation is necessary.

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ORAL PRESENTATION

Liver stiffness measured by Fibroscan is markedly influenced by meal ingestion in healthy volunteers

Abstract Body: Objectives: Evaluation of liver stiffness by Fibroscan® has become widely used in the assessment of the stage of diverse liver diseases. We wanted to evaluate how ingestion of a controlled calorie meal influences liver stiffness measured by Fibroscan® in healthy subjects.

Patients and methods: We evaluated 22 volunteers without known liver or other disease (12 female, 10 male). Liver stiffness was evaluated using Fibroscan® in fasting and 30 minutes after a standard liquid meal of 220 ml containing 330 kcal.

Results: Ingestion of a liquid meal increased liver stiffness in comparison with fasting levels in 19/22 subjects. In fasting mean liver stiffness was $4,73 \pm 1,29$ kPa and after meal $5,65 \pm 1,83$ kPa. Mean difference in fasting and after meal liver stiffness for the 19 subject was $1,23 \pm 1,08$ kPa. Using the Wilcoxon test for paired samples the increase was statistically significant with a $P=0.0003$. This was irrespective of sex, age and fasting liver stiffness measurement. Liver stiffness values returned to baseline fasting levels within two hours.

Conclusion: Liver stiffness increases after ingestion of a meal probably due to increased portal blood flow as observed in our study on a healthy Croatian cohort. The results were irrespective of age, sex and fasting liver stiffness values. We suggest, based on the results, that all liver stiffness measurement should be done in a fasting state.

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ORAL PRESENTATION

PROGNOSTIC VALUE OF SYNDECAN-1 AND SYNDECAN-2 EXPRESSION IN PANCREATIC ADENOCARCINOMA

Objective: The goal of the research was to investigate the correlation between expression of syndecan-1 and syndecan-2 in pancreatic adenocarcinoma and disease prognosis and final outcome. **Methods:** Syndecan-1 and syndecan-2 immunohistochemical expression and its relationship with established prognostic features were assessed in a series of 53 patients with pancreatic ductal adenocarcinoma.

Results: All together 53 patient with pancreatic adenocarcinoma were enrolled in this study. Syndecan-1 was expressed in the epithelia of 48 and syndecan-2 in epithelia of 23 pancreatic adenocarcinomas. The expression of syndecan-1 and syndecan-2 in the stroma of adenocarcinoma was found in 30 samples. From all the analyzed clinical and pathological parameters only age of the patient was significantly statistically correlated with the stromal expression of syndecan-2 ($p=0,014$). Patients who had a stromal expression of syndecan-2 in the carcinoma tissue were significantly older than those without syndecan-2 stromal expression. The differences between carcinomal epithelial ($p=0,444$, $p=0,088$) and stromal ($p=0,421$, $p=0,080$) syndecan-1 and -2 expression did not differ in patients who died from adenocarcinoma and those who were still alive at the end point of follow-up period. No statistically significant correlation was found among syndecan-1 and syndecan-2 epithelial and stromal carcinoma expression ($p=0,858$, $p=0,847$). According to Cox regression analysis, as a statistically significant factor of survival rate arose the epithelial expression of syndecan-2 ($p=0,029$). Patient with over expression of syndecan-2 lived statistically longer that those with a lesser expression.

Conclusion: On the basis of generated data and final results, it was concluded that epithelial and stromal expression of syndecan-1 and syndecan-2 in pancreatic adenocarcinoma could have influence in the carcinogenesis and disease progression. Therefore both analyzed markers could be candidates for the development and implementation of specific molecular therapy. The epithelial expression of syndecan-1 in pancreatic carcinoma revealed it's self as a significant prognostic factor and could have a role as an additional indicator of disease aggressiveness.

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ORAL PRESENTATION

EFFECT OF PENTADECAPEPTIDE BPC 157 ON MONOCROTALINE INDUCED COR PULMONALE IN RAT

Abstract Body: Pentadecapeptide BPC 157 modulates synthesis of NO, has antiarrhythmic effect and protective effect on endothelium. This study was designed to investigate its effects in monocrotaline induced pulmonary hypertension in rats. It included 13 groups by 6 rats (body weight 150-200 g). Pentadecapeptide BPC 157 was given daily, either in drinking water or intraperitoneally, in two different dosages (10 µg/kg i 10 ng/kg), in cotreatment protocol day 1 - 29, while in posttreatment protocol therapeutic intervention with pentadecapeptide BPC 157 was started when the development of pulmonary hypertension had already commenced and was upheld day 15 - 29. In control groups a marked right heart hypertrophy was evident, and massive thickening of the precapillary artery smooth muscle layer was histologically apparent, with cilincal deterioration due to pulmonary hypertension during the 4th week after MCT injection, and some animals dying during this period because of right heart failure. In cotreatment protocol pentadecapeptide BPC 157 consistently prevented in all application modalities the development of pulmonary hypertension with all its manifestations, while in posttreatment therapeutic protocol BPC 157 reversed also in all application modalities the already established PAH. We conclude that pentadecapeptide BPC 157 prevents and attenuates MCT-induced pulmonary hypertension and cor pulmonale in rats.

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ORAL PRESENTATION

ISOPRENALINE INDUCED MYOCARDIAL INFARCTION IN RAT, HARMFUL EFFECT OF L-NAME, BENEFICIAL EFFECT AFTER L-ARGININE AND BPC 157

We focused on counteraction of isoprenaline (75 or 150mg/kg s.c.) induced myocardial infarction (one challenge) and re-infarction (isoprenaline at 0h and 24h, two challenges) in rats (assessed at the end of the subsequent 24h period). BPC 157 (10ng/kg, 10µg/kg i.p.), L-NAME (5mg/kg i.p.), L-arginine (200 mg/kg i.p.) were given alone or together at (i) 30 min before; or, alternatively (ii) at 5 min after isoprenaline. BPC 157 markedly counteracts all isoprenaline induced myocardial alterations: reduced ST elevation/depression in ECG, decreased values of cardiac enzymes and troponin T, markedly improved clinical presentation and macro/microscopic lesion presentation. In addition, RT-PCR demonstrated increased eNOS, iNOS, COX-2 mRNA levels in the heart septum tissue of isoprenaline rats and showed less eNOS and COX-2 mRNA levels in BPC 157 treated rats. L-arginine was also beneficial while L-NAME application further aggravated both isoprenaline lesions. L-arginine brings down aggravation by NOS-blockade with L-NAME to control levels. BPC 157 nullifies the effect of L-NAME and brings all isoprenaline+L-NAME lesions markedly below control values. Higher effects of BPC 157 were not potentiated by L-arginine. Thus, BPC 157 with a particular, rapid and constant effect in all regimens, given before or after isoprenaline may present an interesting therapeutic tool.

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ORAL PRESENTATION

PERCUTANEOUS ENDOSCOPIC GASTROSTOMY – A SINGLE-CENTER EXPERIENCE

Aim: Percutaneous endoscopic gastrostomy (PEG) is a tool of choice for providing long-term enteral feeding to patients with a variety of chronic and degenerative neuromuscular and malignant diseases which cause swallowing disturbances. The aim of this study is to evaluate our single endoscopic center experience with PEG procedure taking into account demographic data, indications, technical problems and related complications.

Materials and methods: The demographic data, complications and follow-up findings of patients who had undergone PEG between April 2013 and April 2016 were examined prospectively using medical files. We have taken into account the recent and long-term complications, the replacement or removal of the tube were recorded, the patients swallowing function were observed.

Results: One hundred twenty seven percutaneous endoscopic gastrostomy and three hundred percutaneous endoscopic gastrostomy related procedures (143 PEG replacements and 157 regular PEG check outs) were performed in 135 adult patients during a three-year period. The median age of the patients was 59 years (range 17-85 years; 88 male gender). Patients with neurological and malignant diseases (head and neck cancer followed by esophageal and stomach cancer) constituted the majority (64.7% and 26.5% respectively). The PEG placement was successful in 97% of cases (3 patients were referred to surgery). During the follow up period overall rate of complications was 26%. Most common minor complications included local stoma site infection (28%), PEG clogging (26%), and granulations (9%) and were successfully treated conservatively; buried bumper syndrome with skin abscess developed in two patients leading to septic complications and death in one of them. In 12 cases PEG tube was removed when patients have recovered their normal swallowing function.

Conclusion: PEG is safe and effective method for enteric nutrition feeding in adults with swallowing disorders. Owing to the lack of sufficient knowledge and the consequent failure to recognize the problems of patients with secondary dysphagia, PEG is still unsatisfactory and underutilized tool for ensuring artificial enteral feeding in today's routine medical practice in Croatia.

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ORAL PRESENTATION

The effect of pentadecapeptide BPC 157 and the role of nitric oxide system on uterus damaged by cysteamine

Objectives: To determine that cysteamine induces damages of endometrium and that pentadecapeptide BPC 157 counteracts those lesions as well as interacts with NO-system.

Materials and methods: Cysteamine was applied directly in each of the uterus horns (40mg/0.5ml/uterus horn), in the total dose of the 400 mg/kg. Effect was evaluated by macroscopic and microscopic examination after 2h, 3days and 7 days. Medication was given after cysteamine, either immediately or 2h later, including pentadecapeptide BPC 157 10µg/kg or 10ng/kg per day, intraperitoneally (5ml/kg), or per-orally (12ml/rat/day, 0.16ug/ml, 0.16ng/ml), L-NAME (5mg/kg/day intraperitoneally), L-arginine (100mg/kg/day intraperitoneally), given alone and/or in combinations, while controls received saline (5ml/kg) intraperitoneally or drinking water (12ml/rat/day).

Results: We demonstrated a strong damaging effect of cysteamine on the endometrium. BPC 157 given intraperitoneally or per-orally prevented lesions development and counteracted already extensive lesions. We proved interaction between the tested pentadecapeptide BPC 157 in conditions of modulation of the NO-synthetase inhibitor L-NAME or NO-synthetase substrate L-arginine. These suggest NO-system related regeneration of the endometrium and beneficial effect of BPC 157 connected with NO-system.

Conclusions: Pentadecapeptide BPC 157 has shown a significant protective effect to the uterus and it has therapeutical potential in this experimental model.

Key words: cysteamine, endometrium, BPC 157, NO-system

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ORAL PRESENTATION

THE EFFECT OF BPC 157 ON ISCHEMIC/REPERFUSION INJURIES IN RAT BRAIN

Introduction. Ischemic/reperfusion injuries are elementary pathophysiological findings in stroke, thereby making it the third most common cause of death in the modern world and the first cause of long-term disability. Pentadecapeptide BPC 157, has already been proven to have an effect on vessel integrity, it is a mediator of Robert's cytoprotection and interacts with the NO system, all of which, make it a promising agent when it comes to cerebral ischemic/reperfusion injuries.

Methods. In this experiment, ischemic/reperfusion injuries are induced using bilateral carotid artery occlusion (BCAO). The effect of BPC 157 on ischemic/reperfusion injuries was investigated in male Wistar Albino rats. After an occlusion of 20 min, the rats were randomly divided into groups. The treated group received BPC 157 (10µg/kg, 10ng/kg, I.P.) right after surgery, while the control group received saline (1ml, I.P.) immediately after surgery. After a reperfusion period of 24 or 72 hours, the neurological assessment was performed and samples were gathered for further examination. Neurological assessment was conducted using the Morrison water maze test (MWMT) and beam walk test (BWT).

Results. In the MWMT the control animals had far greater memory loss and spatial orientation loss, while the BPC 157 treated group had almost no loss in the MWMT. The control group lost 10.3 seconds, while the BPC 157 treated group gained 1 second in comparison to the training results. In the beam walk test, we also observed substantial differences between the control and treated group, where the control group walked far worse and scored 1, while the BPC 157 treated group walked much better and scored 4. The pathology findings concurred with the results obtained in the neurological assessment.

Conclusion. Pentadecapeptide BPC 157 showed that it counteracts ischemic/reperfusion injuries, saving the rats from memory and orientation loss, as well as maintaining their motor capabilities. The results we present here are promising and prove that BPC 157 has potential as a neuroprotective agent in cerebral ischemic/reperfusion injuries, although further investigations should be conducted to further confirm the full effects of BPC 157.

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POSTER PRESENTATION

Antipsychotic-like behavioral effects and cognitive enhancement by a stable gastric pentadecapeptide BPC 157 examined in interaction with NO – agents

BPC 157 interacts with NO-system, and NO plays an important through and contradictory role in psychosis. This may be generally important since impacted by NO-system, antipsychotic-like manifestations, should be simultaneously manipulated in both ways with NO-system activity modulation. Therefore, antipsychotic-like effects of BPC 157 were evaluated in animal models of psychosis in condition of inhibition (L-NAME) and/or stimulation (L-arginine) of NO-system. Antipsychotic-like effects were tested by the ability to reduce amphetamine, apomorphine and MK-801 induced hyperactivity. Catalepsy was assessed by measuring step-down latency. Cognitive impairment was assessed in ketamine-induced performance deficits in the novel object recognition task. Prevention of the induction and the progression of locomotor sensitization induced with intermittent injections of methamphetamine were measured by locomotor activity testing. BPC 157 and L-arginine alone or in combination reduced amphetamine- and apomorphine-induced hyperactivity and MK-801-induced immobility, stereotypy and ataxia. L-NAME and L-NAME/L-arginine reduced amphetamine-induced hyperactivity, and had not effect on apomorphine and MK-801-induced behavioral changes. Contrary, BPC in presence of L-NAME or L-NAME/L-arginine attenuated apomorphine- and amphetamine-induced motor activity, and attenuated behavioral changes caused by MK-801. BPC 157 alone and in combination with L-arginine antagonizes catalepsy produced by haloperidol / potentiated with L-NAME. L-arginine and BPC/L-arginine attenuated catalepsy induced with haloperidol, but L-arginine alone did not prevented haloperidol-catalepsy potentiated with L-NAME. Cognitive impairments induced with ketamine in novel object recognition task could be antagonized by the BPC 157 and the NO donor L-arginine. The cognitive impairment could also be antagonized by BPC 157 in presence of L-NAME or L-arginine or their combination. Cognitive deficit couldn't be reversed by L-NAME alone or with L-arginine in combination with L-NAME. BPC 157 alone or in combination with L-NAME/L-arginine prevented the induction and progression of locomotor sensitization caused by methamphetamine. L-NAME and L-arginine alone did not prevented behavioral sensitization caused with methamphetamine, but in combination with BPC diminish its effect. Thus, such balancing effect of BPC 157 could be important providing a unique perspective of dopamine system regulation, considering the bi-directional nature of dopamine changes in psychiatric disorders. Accumulated information regarding the interactions of nitric oxide and compounds used in experiments, and BPC 157 consistent counteracting effect, indicate that BPC 157 has a behavioral profile consistent with antipsychotic-like efficacy and shows liability to prevent extrapyramidal symptoms. Prevention of the sensitization shows also the potential of BPC 157 in prevention of expressing a disease in risk population for schizophrenia. This might be useful considering the particular background and very safe profile of BPC 157 so far demonstrated. The hypothesis that BPC 157 is effective in treating all symptom domains of schizophrenia is confirmed by preclinical experiments. The model that takes into account the NO-cGMP system in schizophrenia, represents a new approach in the treatment of a disease. The consequence of such a concept is a medication that reinforced the function of NMDA receptors, but also keeps dopamine function. With a confirmation of this hypothesis it meets a significant treatment need in the overall management of this severe and chronic illness.

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POSTER PRESENTATION

The pentadecapeptide BPC 157 counteracts the diclofenac overdose and its impairment of ileoileal anastomosis healing

Aim. We analyzed the effect of the pentadecapeptide BPC 157 onto the healing the ileoileal anastomosis accompanied with the diclofenac overdose.

Methods. Under deep anesthesia 60 male Wistar Albino rats underwent the surgical procedure of creating an ileoileal anastomosis (Surgery Today, 2007). The animals were divided randomly into control groups treated with saline (5mL/kg, i.p.), while 30 animals received BPC 157 (10 µg/kg, i.p.). During operation 45 animals (15 BPC 157 and 15 controls) received an overdose of diclofenac (12.5 mg/kg, i.p.). At the end of each experimental period (2 days, 6 days, 14 days) the animals were sacrificed and the biomechanical, functional, macroscopic and microscopic assessment were performed.

Results. In animals which received diclofenac the healing of anastomosis was impaired in comparison to those which did not receive diclofenac overdose. The animals treated with the pentadecapeptide BPC 157 shown the signs of recovery (larger volume of water needed to cause the anastomotic leakage, lower rate of mortality and obstruction) even at those animals that received the overdose of diclofenac. The raised values of serum AST, ALT were lower in animals treated with the pentadecapeptide BPC 157. The morphological assessment, both macroscopic and microscopic shown the reduced amount of hepatic and gut lesions in animals treated with BPC 157.

Conclusions. BPC 157 initiates and promotes the anastomotic healing despite the diclofenac overdose, which impairs the healing of anatomosis in control animals.

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POSTER PRESENTATION

The healing of colocutaneous fistulas and diclofenac overdose. The pentadecapeptide BPC 157

Aim. We analyzed the impact of diclofenac overdose on the healing of colocutaneous fistulas. Since the pentadecapeptide BPC 157 healed colocutaneous fistulas efficiently (J Pharm Sci, 2008) we suggest its possible effect in condition of healing impaired with diclofenac intoxication.

Methods. 60 male Wistar Albino rats under deep anesthesia underwent surgical procedure. A colocutaneous fistula was created (J Pharm Sci, 2008). During operation 30 animals received an overdose of diclofenac (6.25 mg/kg, i.p.). The animals were randomly assigned into two groups; control group treated with saline (5 mL/kg, i.p.), and group treated with BPC 157 (10 µg/kg). At the end of each experimental period (7 days, 14 days, 28 days) the animals were sacrificed and later biomechanical, functional, macroscopic and microscopic assessment were performed.

Results. In animals that received diclofenac the healing of fistulas was impaired in comparison to those that did not receive the diclofenac overdose. Gradually, the majority of animals treated with the pentadecapeptide BPC 157 started to defecate through anus, while the control animals defecate through fistula. At the seventh postoperative day the control were able to sustain only a small volume of water without leakage through fistula, while the animals treated with BPC 157 sustained larger volume.

Conclusions. Diclofenac impairs healing of colocutaneous fistulas. BPC 157 shows healing effect onto both skin and colonic defect of colocutaneous fistula despite the impairment caused by diclofenac intoxication.

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POSTER PRESENTATION

The healing of ileorectal anastomosis and pentadecapeptide BPC 157

Aim. The pentadecapeptide BPC 157 has already been proved effective at healing ileoileal and jejunioileal anastomosis at short bowel syndrome (SurgToday, 2007 ; Dig Dis Sci, 2009). In this study we wanted to assess whether it is effective at healing ileorectal anastomosis. Another goal would be to see whether BPC 157 recovers animals` state following the subtotal colectomy.

Methods. 60 male Wistar Albino rats under deep anesthesia underwent subtotal colectomy with ileorectal anastomosis formation. The animals were randomly assigned into two groups; half of them were in control group that was treated with saline (5mL/kg, i.p.), while the other received BPC 157 (10 µg/kg). The treatment was once daily, the first dose was applied immediately after the operation and the animals received the last dose 24h before they were sacrificed. The animals were sacrificed 3, 7 and 14 days following the surgery and the biomechanical, functional, macroscopic and microscopic assessment was performed.

Results. In BPC 157 treated group mortality was less, the same was with body weight loss. The ileorectal anastomosis could sustain less volume of water at control animals before leakage started. During all experimental period control animals had diarrhea, while in BPC 157 treated animals the stool gradually became formed.

Conclusions. According to these results BPC 157 enhances the healing of ileorectal anastomosis and reduces the adverse effects of subtotal colectomy.

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POSTER PRESENTATION

The effect of the pentadecapeptide BPC 157 onto colocutaneous fistula healing aggravated by the bile duct ligation

Aim. The pentadecapeptide BPC 157 has shown its effectiveness in healing different organic lesions including newly formed colocutaneous fistulas (J Pharmacol Sci 2008).

Methods. 60 male Wistar Albino rats underwent surgical procedure and a colocutaneous fistula was created (J Pharm Sci, 2008). After that the bile duct ligation was performed in 30 rats in order to cause bile duct obstruction. Animals were randomly assigned into two groups: control -saline (5mL/kg, i.p.), BPC 157 (10 µg/kg). Biomechanical, functional, macroscopic and microscopic assessment was performed.

Results. In animals that underwent the bile duct ligation the healing of fistulas was impaired. On the second postoperative day the majority of animals treated with the pentadecapeptide BPC 157 started to defecate through anus, while the control animals defecate through fistula. At the sixth postoperative day the control were able to sustain only a small volume of water without leakage through fistula, while the animals treated with BPC 157 sustained larger volume. At animals treated with BPC 157 diameters of fistula were diminished, at both external and internal sides of fistula. In controls the liver enzymes were raised and the fistula healing was impaired. An interesting issue is the in BPC 157 treated animals the fistula closed faster than in those without bile duct ligation.

Conclusions. BPC 157 provides sufficient healing of colocutaneous fistulas and reduced of liver enzymes previously caused by bile duct ligation. The results points out the pentadecapeptide as a possible agent of adaptive cytoprotection.

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POSTER PRESENTATION

THE EFFECT OF PENTADECAPEPTIDE BPC 157 ON PLATELET AGGREGATION IN RATS TREATED WITH ASPIRIN, CLOPIDOGREL AND CILOSTAZOL

INTRODUCTION: Prevention of ischemic events due to formation of an occlusive thrombus is based on the effective inhibition of platelets. Individual variability of response to therapy and resistance to antithrombotics sometimes requires different drugs to be combined in order to achieve their cumulative effect, often resulting in an increased risk for bleeding. BPC 157 has been proven efficient in preventing occlusive thrombus formation after the creation of an abdominal aorta anastomosis and in shortening of bleeding time after amputation in rats treated with aspirine.

OBJECTIVE: The study was designed to determine the effect of BPC 157 on platelet aggregation in rats treated with aspirine (ASK), clopidogrel (CLO) or cilostazol (CILO) measured by multiple electrode aggregometry (MEA).

MATERIAL AND METHODS: Sixty male Wistar rats were divided into 3 groups treated with ASK 10 mg/kg, CLO 10 mg/kg or CILO 10 mg/kg. Each group was divided into two subgroups (n=10) receiving 2 ml of tap water (control) or BPC 157 10 µg/kg dissolved in 2 ml tap water, immediately after designated antithrombotic was applied. All drugs and BPC 157 were administered intragastrically for three consecutive days. Blood samples were drawn through intracardiac puncture into lithium heparine tubes. Platelet aggregation in response to arachidonic acid (AA), adenosine diphosphate (ADP), collagen and AA/PGE combination was assessed with MEA and expressed as area under curve (AUC).

RESULTS: Compared to control animals, rats receiving BPC 157 exhibited significant improvement in platelet aggregation in response to AA (mean 53.2 +/- 4.44 vs 58.6 +/- 2.41 AUC, p= 0.026) after the application of ASK and in response to ADP (mean 41.4 +/- 11.7 vs 62.6 +/- 9.61 AUC, p=0.007) after the application of CLO. Surprisingly, control animals treated with cilostazol initially demonstrated higher degree of aggregation in response to examined agonists compared to normal values, but return to lower values in response to AA (mean 70.2 +/- 7.16 vs 56.8 +/- 6.26 AUC, p=0.007) and AA/PGE combination (mean 70 +/- 5.83 vs 55 +/- 5.87 AUC, p=0.002) in animals receiving BPC 157.

CONCLUSION: BPC 157 significantly improves inhibited platelet aggregation in response to AA in rats treated with ASK, in response to ADP in rats treated with clopidogrel, and lowers increased aggregation in response to AA and AA/PGE combination in rats treated with cilostazol, measured by MEA.

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POSTER PRESENTATION

Pentadecapeptide BPC 157 and infrarenal aortic coarctation

Aim. An aortic coarctation is a complex clinical and pathological item. Such state causes multiple organic lesions, especially unless it is treated surgically. The pentadecapeptide BPC 157 has already shown an efficient effect upon ischemic tissue healing. Therefore we assessed its effect onto the possible reduction of ischemic damage of the myocardial and limb muscular tissue the same as its effect to the blood pressure values.

Methods. 50 Male Wistar Albino rats had been anesthetized and then they underwent surgical procedure. The artificial stenosis of the abdominal aorta was made between two renal arteries. The animals were assigned into five groups control group (treated with saline 4 mL/kg). 2 groups treated with pentadecapeptide BPC 157 10 µg/kg; one group started receiving the treatment immediately after the surgery, while the second started to receive therapy a month following the surgery. On the other hand another 2 groups received the pentadecapeptide BPC 157 at the doses of 10 ng/kg; one group started therapy immediately after surgery, while the other started a month after. The walking pattern, the motor functional index, ECG changes, and blood pressure changes were assessed.

Results. The BPC 157 treated animals developed an early musculoskeletal structural and functional recovery of the inferior limbs, while the control animals continuously weakened. The ECG has shown the left ventricular hypertrophy formation which was absent in BPC 157 treated animals.

Conclusion. The pentadecapeptide BPC 157 has reduced efficiently the structural and functional changes caused by aortic coarctation.

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POSTER PRESENTATION

Pentadecapeptide BPC 157 heals colovesical lesions in rats

Aim. The healing of internal fistulas such as colovesical is particular problem in fistulas healing. The stable gastric pentadecapeptide BPC 157, tested in clinical trials for IBD therapy (Curr Pharm Des 2011; Curr Med Chem 2012;19:126-32), already proved effective in healing external fistulas (J Pharmacol Sci 2008; Dig Dis Sci 2009). BPC 157 may be a successful therapy of colovesical fistulas (European J Pharm, 2016). Single local application of cysteamine intravesically induces inflammation that comprises ulceration, necrotic area formation along with the urethral sphincter pressure failure. The inflammation usually does not limit just onto the area of urinary bladder. Very often it is complicated with the ulceration of the urinary bladder wall and the pouring out the cysteamine into the pelvis. Therefore the inflammation spreads and the pelvic structures damage and form adhesive complexes.

Methods . The injury was caused upon the male Wistar Albino rats, by applying the 400mg/kg cysteamine soluted in distilled water directly into the urinary bladder. The cysteamine was applied through needle that was inserted transluminally on previously anesthetized rats. The 30 animals were assigned into three groups of ten animals. Half of them received the pentadecapeptide BPC 157 (10µg/kg i.p.) as therapy, while the others received the equivalent volume of the saline (4mL/kg). Each group was sacrificed at the end of the proper time interval (7,14 days).

Results. The control animals developed the failure of the urethral sphincter pressure, the same as the widespread inflammation, along with ulcerative and necrotic changes of the urinary bladder. The BPC 157 treated group has shown the recovery that could have been confirmed macroscopically, microscopically and functionally. In addition the BPC 157 animals the urethral sphincter has shown the function recovery through the values of sphincter pressure that were close to those in normal healthy animals.

Conclusion. The BPC 157 has recovered the urethral sphincter pressure failure and healed the inflammatory changes of the inflamed urinary bladder wall.

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POSTER PRESENTATION

The cysteamine colitis model in rats elucidates the healing effect of pentadecapeptide BPC 157

Aim. The pentadecapeptide BPC 157 has already been proved effective in healing of ileoileal anastomosis (Surg Today. 2007), colon-colon anastomosis (Cell injury and protection in the GI tract, 1997), colocutaneous fistulas (J Pharmacol Sci. 2008) and the cysteamine induced colitis (J Physiol Paris. 2001). It is also proved effective onto the healing of the colon-colon anastomosis complicated by the cysteamine induced colitis in rats (J Physiol and Pharmacol.2013)

Methods. Under deep anesthesia the cysteamine was applied through enema at 8 cm proximal to the anus and anastomosis was created 5 cm proximal to anus. The 40 animals were randomly assigned into control group treated with saline (4mL/kg) and groups treated with pentadecapeptide BPC 157 (10 µg/kg). The animals were treated once daily and assessed at the end of experimental period (3, 5, 7, 14 days) macroscopically, microscopically, biomechanically (the bursting pressure and the anal sphincter pressure). We performed assessment of the passage of the fecal stream and the presence of the retention and obstruction. The consistence of stool and the body mass were noted.

Results. The animals treated with the BPC 157 have shown the milder body weight loss, the higher survival rate, formed feces 3 days following surgery and the absence of passage impairment. The anastomosis in controls healed poorly, leaked at the lower bursting pressure. Along with the morphological restitution the functional recovery presented.

Conclusions. The pentadecapeptide BPC 157 brought to the efficient healing of both the anastomosis and the inflammatory damaged bowel, which was not achieved at the controls even at the longest experimental period.

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POSTER PRESENTATION

Pentadecapeptide BPC 157 and chronic skin and bowel lesions. The impact on healing of the colocutaneous fistulas model in rats. Short term and long term follow up

Aim. The pentadecapeptide BPC 157 has shown its effectiveness in healing different organic lesions including newly formed colocutaneous fistulas (J Pharmacol Sci 2008). In this study we assessed its activity onto the chronic lesions which cannot heal spontaneously. We performed a 16 months long follow up in order to estimate the incidence of possible recurrences.

Methods. A colocutaneous fistula has been created according to previously described model (J Pharmacol Sci 2008) at 100 male Wistar Albino rats. After the 4 weeks period without any therapy, the animals whose fistula healed spontaneously were excluded (40/100). Others were randomly assigned into 3 groups of 20 animals: the control group which started to receive the saline (5mL/kg, i.p.). Two other groups started to receive BPC 157 (10 µg/kg i.p.; 10 ng/kg i.p.) for 4 weeks. The animals were followed up through another 16 months and then sacrificed and assessed (J Pharmacol Sci 2008).

Results. Animals treated with BPC 157 started to expose the morphological and functional recovery from the 3rd post treatment day and through 2 weeks of treatment fistulas were reduced significantly and animals started gradually to defecate only through anus. In control animals fistula remained and they defecated through it. Animals treated with BPC 157 did not develop recurrences during whole follow up period of 16 months. In addition, during 16 months of follow up at control animals none of the fistulas closed.

Conclusions. BPC 157 heals both chronic and newly formed colocutaneous fistulas and prevents recurrences when the morphological restitution is achieved.

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POSTER PRESENTATION

THE ROLE OF PENTADECAPEPTIDE BPC 157 ON INCISIONAL VENTRAL HERNIA PREVENTION AND WOUND TENSILE STRENGTH IMPACT

Introduction: Pentadecapeptide BPC 157 has proven positive effect on skin wounds and muscle-tendon damage healing, but its effect on laparotomy wounds, incisional hernia prevention as well as wound tensile strength impact has not yet been studied.

Materials and methods: Ingrained method (described by Dubay, 2004.) of early wound failure induction (satisfactory hernia incidence) was performed on Wistar rats. According to experiment protocol rats in experimental groups (BPC157mcg2w, BPC157mcg3w and BPC157mcg4w) were treated with BPC 157 (10mcg/kg, 0.16mcg/mL, 12mL/day/rat, per os in drinking water), while control group received drinking water only. Rats in BPC157mcg2w group were sacrificed 14 days, BPC157mcg3w in 21 days, and BPC157mcg4w group 28 days after initial operation. Incisional hernia presence was monitored. 5mm broad samples (no thickness difference, $p=0.875$) were taken from the proximal part of laparotomy for tensile strength measurement and analyzed on Beta 50-5 (Messphysik, Austria) Testing Machine. Cutaneous wound healing quality and intraabdominal adhesions were rated (our ordinary scales used).

Results: In Control group 73.3% of rats developed hernia while 20% of rats in all experimental groups showed hernia presence. Hernia width in BPC157mcg2w group and BPC157mcg3w group was not significantly different from hernia width in Control group ($p=0.126$; $p=0.072$), while hernia width in BPC157mcg4w was significantly smaller ($p=0.049$). BPC157mcg2w and BPC157mcg3w groups tensile strength was not significantly different ($p=0.679$; $p=0.734$) while BPC157mcg4w tensile strength showed significant difference to Control group ($p=0.073$, p value 0.1 taken significant because of few non-correctable factors). There was a significant difference in cutaneous wound healing ($p=0.024$) and adhesions presence ($P=0.031$) between Control group and BPC 157 treated animals.

Discussion: Pentadecapeptide BPC 157 has evident hernia prevention, cutaneous wound healing and intraabdominal adhesions prevention effect. No hernia size difference in groups monitored for 14 and 21 days versus significant hernia size difference in 28 days monitored animals pinpoints over time hernia growth prevented by BPC 157. Tensile strength significant difference in 28 days monitored group also implies prolonged BPC 157 healing impact, through all three wound healing phases.

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POSTER PRESENTATION

PENTADECAPEPTIDE BPC 157, ANTICOAGULANTS, NO SYSTEM - THE EFFECTS ON HEMOSTATIC PARAMETERS

BPC 157 is a stable gastric pentadecapeptide recently implicated with a role in haemostasis. While NO is largely implicated in haemostatic mechanisms, in tail-amputation-models under anticoagulant-administration, both the NO-synthase (NOS)-blocker, L-NAME (prothrombotic) and the NOS-substrate L-arginine (antithrombotic), were little investigated.

This study determines the effect of the pentadecapeptide BPC157 on the values of hemostatic parameters in rats after administration of anticoagulants (heparin, aspirin and warfarin). Also, this study extends and clarifies the effect of BPC157 on hemostasis with addition of N(G)-nitro-L-arginine methylester (L-NAME) and L-arginine in heparin and warfarin treated rats.

Male Albino Wistar rats were used in all of the experiments (approved by the Local Ethics Committee). Tail amputation, and/or i.v.-heparin, i.g.-warfarin, i.g./i.p.-aspirin, were used in rats. Treatment includes BPC 157, L-NAME, L-arginine, per se and their combination. Platelet count, hematocrit, prothrombin time, active partial thromboplastin time, thrombin time, fibrinogen and bleeding, were measured to evaluate the haemostatic effect of the agents.

In rats, BPC 157 (10 µg/kg, 10 ng/kg) improved always reduced bleeding time and amount of bleeding after (tail) amputation only, heparin (250 mg/kg, 25mg/kg, 10mg/kg i.v.), warfarin (1.5mg/kg i.g. once daily for 3 consecutive days), aspirin (0.1g/kg i.g. once daily/3 consecutive days or 1.0 g/kg i.p. once). All heparin-, warfarin-, and aspirin-rats and normal-rats that received BPC 157 exhibited lesser fall in platelets count. BPC 157 attenuated over-increased APTT-, TT-values in 10mg/kg heparin-rats, but did not influence heparin activity (anti-Xa test). Furthermore, we investigated the effects of BPC 157 on the L-NAME and L-arginine-induced haemostatic actions under different pathological condition (tail amputation without or with anticoagulants: heparin or warfarin). As for L-NAME and/or L-arginine, we noted: L-arginine (100 mg/kg i.p.)-rats: more bleeding, less/no thrombocytopenia; L-NAME (5 mg/kg i.p.)-rats: less bleeding (amputation only), but present thrombocytopenia; L-NAME+L-arginine-rats also exhibited thrombocytopenia: L-NAME counteracted L-arginine-increased bleeding, L-arginine did not counteract L-NAME-thrombocytopenia. All animals receiving BPC 157 (BPC 157µg+L-NAME; BPC 157µg+L-arginine, BPC 157µg+L-NAME+L-arginine), exhibited decreased haemorrhage and markedly counteracted thrombocytopenia.

Therefore, we conclude that the application of BPC 157 has effect on hemostasis, reduces bleeding, reduces blood loss, and thrombocytopenia in treated rats regardless of the manner of its application. L-NAME (thrombocytopenia), L-arginine (increased haemorrhage) counteraction and BPC 157 (decreased haemorrhage, counteracted thrombocytopenia) with rescue against different anticoagulants, implicate a BPC 157 modulatory and balancing role with rescued NO-haemostatic mechanisms.

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POSTER PRESENTATION

BPC 157 AND ACUTE OBSTRUCTION OF THE ABDOMINAL AORTA

Introduction: Positive activity of pentadecapeptide BPC 157 on rapid development of collateral circulation may be the main factor for many protective effects against congestion and ischemia in various tissues. We explored the effect of BPC 157 on rapid activation of blood vessels/ anastomosis in severe condition of complete obstruction of abdominal aorta.

Materials and methods: Female Albino rats weighing 350g were used. Animals were randomized to control and pentadecapeptide BPC 157 treated group. Deeply anaesthetised (Ketamine 0.5-4 mg/kg i.p., Normabel 0.04-0.2 mg/kg i.p.) rats underwent abdominal aorta ligation with surgical suture at the infrarenal or bifurcation level. After the operation, the animals received BPC 157 dissolved in saline at a dose of 10 µg/ kg as a local bath (2 ml/rat). Controls received an equivalent volume of 0.9% NaCl in the same way as mentioned above. Invasive arterial blood pressure measurement was performed with cannula (BD Neoflon™ Cannula) connected to a pressure transducer (78534C MONITOR/ TERMINAL Hewlett Packard) inserted in the abdominal aorta above ligature (at the bifurcation level or infrarenal level) 1h after ligation. After 1h interval we analyzed the redistribution of collateral circulation using digital subtraction angiography (DSA).

Results: Control group (infrarenal level) at 1h had the blood pressure in abdominal aorta of 65.7 ± 3.5 mmHg. Control group (bifurcation level) at 1h had the blood pressure in abdominal aorta of 85.3 ± 2.3 mmHg. BPC 157 treated group (infrarenal level) at 1h had pressure in abdominal aorta of 96 ± 3.5 mmHg. BPC 157 treated group (bifurcation level) at 1h had pressure in abdominal aorta of 98.7 ± 2.7 mmHg. In both control groups no redistribution of collateral circulation at 1h interval using digital subtraction angiography (DSA) was observed, while in both BPC 157 treated groups redistribution of collateral circulation (i.e. via renal arteries) was observed.

Conclusion: Pentadecapeptide BPC 157 used intraperitoneally counteracts the effects of acute obstruction of abdominal aorta infrarenally or at bifurcation level.

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POSTER PRESENTATION

THE EFFECT OF PENTADECAPEPTIDE BPC 157 ON EPISCLERAL VEIN CAUTERIZATION MODEL IN RATS

OBJECTIVE OF THE STUDY. Pentadecapeptide BPC 157, given locally or systemically, counteracts atropine mydriasis in rats, while by itself does not affect normal pupil diameter. This study aimed to evaluate the effect of BPC 157 on glaucoma-like features in the rat episcleral vein cauterization model.

METHODS. Albino Wistar rats 200g were used. Under deep anesthesia (Ketamine-HCl 50-60 mg/kg + Xylazine-HCl 5-10 mg/kg intraperitoneally) and Tetrakain 0.5% drops, two dorsal episcleral veins and one temporal episcleral vein were isolated. A cautery was specifically applied to the selected vein. Intraperitoneal medication (pentadecapeptide BPC 157(10µg/kg); saline 5ml/kg) was applied either immediately after surgery (early treatment), or at 24h thereafter (delayed treatment). Early treatment rats received treatment immediately after surgery while alternatively delayed treatment received at 24h after. Controls received an equivolume of 0.9%NaCl (5ml/kg) intraperitoneally. Intraocular pressure (IOP) (% of normal IOP), pupillar function were measured at 24h and 48h after surgery. Non-invasive IOP measurements were taken after local application of anesthetic, by aplanation tonometer Tonopen XL. Pupillar function was photographed by USB Veho Discovery VMS-004 Deluxe microcamera. Photographs taken before and after application of BPC 157 or saline were analyzed with special software bought with camera for measurement of pupillar diameter (r =mm), range (C =mm) and surface (S =mm²). Vascularization of the eye fundus and presentation of optic nerve papilla were analyzed with microcamera and Digital Widefield lupe 90D at 4 weeks after procedure. The vessels extending into and out of the optic disc were particularly analyzed. Retinal changes alterations in vessel caliber and tortuosity, optic disc pallor and leakage of the retinal arterioles and venules were scored (1-3).

RESULTS. Early treatment. At 24 hours after surgery control rats exhibited increased values ($r=1.3\pm 0.1$, $C=7.3\pm 0.4$, $S=6.0\pm 0.2$, IOP=135%). These increased values were fully counteracted in BPC 157-rats ($r=0.14\pm 0.03$, $C=1\pm 0.05$, $S=0.1\pm 0.03$, IOP=105%).

Delayed treatment. In other experiments, after 24h post-surgery($r=1.4\pm 0.2$, $C=8.3\pm 0.3$, $S=7.0\pm 0.4$, IOP 135%) received medication saline or BPC 157 intraperitoneally. In controls the increased values and failure persisted unchanged ($r=1.4\pm 0.2$, $C=8.3\pm 0.3$, $S=7.0\pm 0.4$, IOP 135%) for next 24h, i.e. till the 48h after surgery. On the other hand, these were counteracted by subsequent administration of BPC 157 as evidenced in next 24h, at 48h post-surgery ($r=0.26\pm 0.04$. $C=1.6\pm 0.1$. $S=0.42\pm 0.05$, IOP=104%).

In all BPC 157 treated animals there was only slight generalized vessel caliber irregularity (difference in diameter between arteries and veins) with a vaguely atrophic optic disc and area not bigger than 1 optic disc diameter containing leaking of retinal vessels (score 1). In control animals there was either moderate generalized vessel caliber irregularity (bigger difference in diameter between arteries and veins with moderate overfilling of veins) with moderate atrophic optic discs and an area ranging between 1-3 optic disc diameters containing leaking of retinal vessels (score 2) or severe generalized vessel caliber irregularity (very big difference in diameter between arteries and veins with severe overfilling of veins) with severe atrophic optic disc and an area more than 3 optic

disc diameters with leaking of retinal vessels (score 3).

CONCLUSION. Episcleral vein cauterization in rats lead to permanently raised intraocular pressure, disabled pupillar function, damaged vascularization of the eye fundus and presentation of optic nerve papilla. Using pentadecapeptide BPC 157, all these pitfalls could be both prevented (development counteracted, BPC 157 given immediately after injury induction), and reversed (BPC 157 given after preexisting injury).

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POSTER PRESENTATION

The effect of applying pentadecapeptide BPC 157 in muscle atrophy after resection of patellar ligament in a rat model

We observed pentadecapeptide BPC 157, L-NAME and L Arginine effects on quadriceps muscle in 3 month old Wistar male rats after patellar ligament resection and following muscle atrophy onset. **Materials and Methods:** After surgical resection of rear right leg patellar ligament in rats, we divided them in 8 groups of 10 rats and studied muscular atrophy development by treating them with pentadecapeptide BPC 157 (20 µg/kg), L- NAME (5 mg/kg), , L Arginine (100 mg/kg), single and combined substance treatment, intraperitoneal application, once a day, treatment duration 14 days. We measured their effect through clinical/functional tests and histological muscle tissue analysis. **Results:** By measuring muscle contracture, muscle strength reduction and muscle diameter reduction we came to results showing that BPC 157 prevents development of muscle contracture, muscle strength reduction and muscle diameter reduction. L arginine decreases development of muscle contracture, muscle strength reduction and muscle diameter reduction compared with control group. In the group receiving L-NAME faster development of muscle contracture and muscle strength reduction was observed alongside larger muscle diameter reduction compared with the control group. Combining two substances was observed BPC 157 and L Arginine resulting in no muscle contracture development and no muscle diameter reduction, while combining BPC 157 and L-NAME antagonistic effect develops leading to minor muscle contracture, muscle strength reduction and muscle diameter reduction compared to control group. In the group combining L-NAME + L Arginine antagonistic effect also develops leading to partial muscle contracture, muscle strength reduction and muscle diameter reduction. Group receiving all 3 substances observed minor muscle contracture, muscle strength reduction and muscle diameter reduction. **Conclusion:** Pentadecapeptide BPC 157 prevents muscle atrophy onset, prevents muscle contracture and enhances muscle reparation in rats.

Keywords: muscle atrophy, muscle contracture, reduced muscle strength, pentadecapeptide BPC 157, L NAME, L Arginine

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POSTER PRESENTATION

In vitro antibacterial activity of pentadecapeptide BPC 157 on *Staphylococcus aureus* and *Escherichia coli*

Introduction

The aim of this study was to examine the in vitro antibacterial activity of pentadecapeptide BPC 157 on bacterial strains of *Staphylococcus aureus* and *Escherichia coli* which were isolated from different clinical specimens and their interaction with conventional antibiotics.

Materials and Methods. In this study was used pentadecapeptide BPC 157, manufactured by Diagen d.o.o., Ljubljana, Slovenia-99 % purity which is dissolved in saline solution and prepared in different concentrations. It was examined the effect of 20 strains: ten strains of *Staphylococcus aureus* (including methicillin resistant *Staphylococcus aureus*- MRSA), and ten strains of *Escherichia coli* (involving ESBL-producing *Escherichia coli*). *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used as control strains. The antibacterial activity of pentadecapeptide BPC 157 was tested by microdilution method (CLSI document M100-S17, CLSI, Wayne, Pennsylvania, USA, 2007). Sensitivity of microorganisms to conventional antibiotics was tested according to standard disk diffusion and microdilution method (CLSI M100-S17 documents, CLSI, Wayne, Pennsylvania, USA, 2007). For *S. aureus* we used ampicillin, vancomycin, gentamycin, and for *E. coli* amikacin, ceftazidime and imipenem. This synergistic action was tested by a combination of disc-diffusion test (M2-A9 of the Clinical and Laboratory Standards Institute (CLSI), USA) and the broth microdilution method. On antibiotic disc, which were used in disk diffusion test, there were added 20 µl of pentadecapeptide BPC 157 in different concentrations (512 µg /ml; 51.2 µg /ml; 5.12 µg /ml and 0.5 µg /ml). Control disk was 20 µl of saline solution. Differences between zones of inhibition with pentadecapeptide BPC 157 and without pentadecapeptide BPC 157 were measured by ruler (in mm), and statistically analyzed. Also, there was determined the minimum inhibitory concentration of antibiotics with the addition of pentadecapeptide BPC 157 on tested bacterial strains. All tests were performed in triplicate. Statistical analysis was done by Friedman and Wilcoxon test.

Results. Sensitivity of isolated strains from clinical material and ATCC strains on pentadecapeptide BPC 157 observed by microdilution method and in the determination of the synergistic activity of pentadecapeptide BPC 157 with antibiotics combined disk-diffusion test, did not show statistically significant difference ($p > 0.950$). Synergistic effects of pentadecapeptide BPC 157 with antibiotics observed by microdilution method, revealed statistically significant difference of *S. aureus* strains who were exposed to vancomycin and pentadecapeptide BPC 157 together ($p = 0.039$). No statistically significant difference was between other tested antibiotic and bacterial strains.

Conclusion. The results of this study suggest the necessity for further testing antibacterial activity of pentadecapeptide BPC 157 with a large number of bacterial strains in combination with a different

type of antibiotics.

Keywords

pentadecapeptide BPC 157, in vitro, Staphylococcus aureus, Escherichia coli

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POSTER PRESENTATION

Effect of retrobulbar application pentadecapeptid BPC 157 on effect of retrobulbar application L-NAME in rats

Female Wistar rats, 200 g, randomly assigned, were treated retrobulbarly with: L-NAME (0.1ml/0.2mg/eye) and BPC 157 (0.1ml/0.2µg/eye 20 minutes after L-NAME application); L-NAME (0.1ml/0.2mg/eye) and BPC 157 (0.1ml/0.2ng/eye 20 minutes after L-NAME); L-NAME (0.1ml/0.2 mg/eye) and 0.9% NaCl (0.1ml/eye 20 minutes after L-NAME application).

Control animals simultaneously received retrobulbar equivolume of 0.9% NaCl.

With a USB microscope camera „Veho discovery VMS-004 deluxe“ and "VOLK" digital wide field loupe for indirect ophthalmoscope eye fundus was recorded in the following time periods: before retrobulbar application of L-NAME (or 0.9% NaCl); 20 minutes after L-NAME (or 0.9% NaCl); again after BPC 157 (or 0.9% NaCl), and 20 minutes after. Furthermore, recording was performed at the post-application day 1., 2., and 7., 14., 28. (last day, just before the sacrifice).

The fundus were analysed by photo analysis software and by histopathologic eyeball analysis on standard sections, paraffin embedded and hemalaun-eosin stained.

The animals behavior was filmed, observing the following parameters: time of immobility (no movement period) and reaction time to visual stimuli (blue-green glove).

The retrobulbar application of L-NAME causes the vasoconstriction of the retina's arteries, ischemia and following animals immobility due to significant visual impairment, while retrobulbar application of BPC 157 leads to retinal ischemia recovery, prevents its atrophy and restores normal animal behavior.

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POSTER PRESENTATION

Atropine, pilocarpine, NO system, BPC 157 on mydriasis in rats and guinea pigs

In living rats' pupil after either eye drops or systemic administration (intraperitoneal), we revealed particular modulatory effects after gastric pentadecapeptide BPC 157 (10 μ g, 10ng, 10pg/kg) as well as a common long-standing (cc 3h) miotic effect after L-NAME (5mg/kg) or L-arginine (100mg/kg), and atropine-mydriasis (2 drops of 1% atropine/eye, agents at the maximal atropine-mydriasis) and pilocarpine miosis and mydriasis (2 drops of 1% atropine/eye, agents together or in posttreatment) sensitivity to NO-system blockade and/or stimulation.

In general, all these agents might counteract atropine-mydriasis and pilocarpine-mydriasis, and thereby, L-NAME-miosis, L-arginine-miosis and pilocarpine-miosis were NO-sensitive. L-NAME and L-arginine miotic effects were mostly parallel (more effectiveness when applied locally and combined), but competitive (providing after intraperitoneal administration sooner returning to normal pupil size in L-NAME+L-arginine-rats).

Pupil radius of the Albino Wistar rats were evaluated by photographing eyes in earlier set intervals by USB microscope camera „Veho discovery VMS-004 deluxe“. Picture processing was done by special measurement software bought with camera.

Applied locally or systemically, modulatory BPC 157 counteracting potential characterizes no influence on normal pupils when given alone while BPC 157 affects induced both miosis and mydriasis. Locally, in rat with normal pupil, BPC 157 augments the miotic effect of L-arginine, counteracts the miotic effect of L-NAME; in counteracting atropine-mydriasis, besides own counteracting potential, BPC 157 has an additive effect with L-arginine, with L-NAME and with L-NAME+L-arginine. Intraperitoneally, BPC 157 shortens the miotic effects of intraperitoneal L-arginine, L-NAME, and L-arginine+L-NAME and does not affect their counteracting effect on atropine-mydriasis. Also, BPC 157 counteract pilocarpine-miosis (shortens time and maximal mydriasis) and pilocarpine mydriasis (shorter duration).

Concluding, the atropine-mydriasis depends on NO-related mechanisms in a particular way and both L-NAME, a NOS-blocker and L-arginine, a NOS-substrate, exhibit a counteraction and miosis (that could be mutually counteracted), an effect thus far undescribed; the atropine-mydriasis, L-NAME-miosis and L-arginine-miosis, may consequently be counteracted by BPC 157 due to its interactions with the NO-system and sphincter function.

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POSTER PRESENTATION

THE EFFECT OF PENTADECAPEPTIDE BPC 157 UPON SUCCYNILCHOLINE AND NO SYSTEM

Introduction: We tested in living rats the hypothesis that the succinylcholine local and systemic threatening effect should depend on NO-related mechanisms in a particular way, and that both N (G) - Nitro - L - arginine methyl ester (L - NAME), NOS - blocker, and L - arginine, NOS - substrate, would both exhibit an aggravating effect in rat, so far not described, and that these succinylcholine - effects, and L - NAME - aggravation and L - arginine - aggravation, may be consequently counteracted by stable gastric pentadecapeptide BPC 157 due to its interactions with NO system, muscle and nerve protection.

Materials and Methods. Medication (/kg IP) (BPC 157 10 μ g, 10 ng, L - NAME 5mg, L - arginine 100mg, saline 5ml) was at 15 min before succinylcholine (1mg/kg IM, 0.2ml/rat anterior tibial muscle) and sacrifice at 4th min (time of death of L - arginine - succinylcholine rats).

Results. Commonly, with BPC 157 succinylcholine course was mitigated, both local and systemic succinylcholine disturbances. BPC 157 completely eliminated hyperkalemia, and arrhythmias, decreased serum - enzymes values, and markedly attenuated or eliminated behavioral agitation, muscle twitches, motionless laying. No violent screaming upon light touch appeared in IM succinylcholine-rats. Accordingly, since very beginning, BPC 157 eliminated leg contracture, counteracted muscle fibers decrease and edema that otherwise appeared in injected and non - injected anterior tibial muscle. These were all aggravated by L- NAME and by L - arginine (leading to rapid death) and counteracted by BPC 157 coadministration.

Conclusion. Succinylcholine, both local and systemic threatening effect depend on NO-related mechanisms in a particular way, since both L - NAME, NOS - blocker and L - arginine, NOS - substrate, exhibit an aggravating effect in rat, and these succinylcholine - effects, and L - NAME - aggravation and L - arginine - aggravation, are consequently counteracted by stable gastric pentadecapeptide BPC 157.

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POSTER PRESENTATION

Cyclophosphamide-induced hemorrhagic cystitis as a particular NO-system disturbance, stable gastric pentadecapeptide BPC 157, L-arginine, versus L-NAME

We focused on cyclophosphamide (100 mg/kg/day intraperitoneally throughout three days) induced hemorrhagic cystitis as a particular nitric oxide (NO)-system disturbance, and therapy possibilities, and thereby that may be attenuated by subsequent administration of NOS substrate L-arginine (100mg/kg/day intraperitoneally), aggravated by NOS-blocker L-NAME (5mg/kg/day intraperitoneally), all influenced by the stable gastric pentadecapeptide BPC 157 ((10 µg/kg/day, 10 ng/kg/day, intraperitoneally or perorally, in drinking water). Regularly, cyclophosphamide dose- and time-dependently increased severe hemorrhagic cystitis lesions, gross lesions, and corresponding urothelial necrosis, vesical edema, erosion, hemorrhage, inflammation, and ulceration, microscopically. The bladder wet weight dramatically increased. Functionally, increased leak point pressure was consistently noted already after first cyclophosphamide administration. Till the second cyclophosphamide administration, L-arginine consistently attenuated regular cyclophosphamide induced severe hemorrhagic cystitis lesions, grossly and microscopically, but not functionally. L-NAME aggravated these lesions and eradicated beneficial effect of L-arginine when combined. BPC 157 administration after cyclophosphamide, given in either dose or in either regimen markedly attenuated all cyclophosphamide lesions, grossly, microscopically. The increase of the bladder wet weight was consistently attenuated. Functionally, increased leak point pressure was reversed to the values noted in normal rats. The similar findings were noted in rats that received BPC 157 together with L-NAME or L-arginine, given alone or combined. Thus, we argue lesions NO-related based on the administration of L-NAME as well as administration of L-arginine, and their mutual interaction, and counteraction by BPC 157 application. Likewise, we reveal new therapeutic possibilities, arguing stable gastric pentadecapeptide BPC 157 and L-arginine, versus L-NAME in rats underwent cyclophosphamide-cystitis.

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POSTER PRESENTATION

EFFECTS OF PENTADECAPEPTIDE BPC 157 ON AMINOGLYCOSIDE NEPHROTOXICITY IN THE RATS

Introduction: we are investigating the effect of pentadecapeptide BPC 157 (hereinafter BPC 157) on aminoglycoside nephrotoxicity (AN) in the rats after application of high dose of gentamicin. We assume that BPC 157 is causing nephroprotective effect by modulating overstimulative activity of iNOS in AN. Therefore we have been applying various combinations of L-NAME and L-arginin with BPC 157 and gentamicin.

Materials and methods: We have been using male Wistar albino rats, body weight 150-200 grams. Rats have been randomized in 17 groups, 10 rats per group. Gentamicin in dose 100 mg/kg of body weight have been administered to the rats in all groups. BPC 157 in attributable groups (AG) has been administered intraperitoneally (10 µg/kg and 10 ng/kg) and perorally (0,16 µg/ml and 0,16 ng/ml at 12 ml water ad libitum per day) in AG. L-NAME (5 mg/kg i.p.), L-arginin (200 mg/kg i.p.) and adequate combination have been administered in AG.

Results: Clinical appearance have been measured and scored (1 – minimal, 2 – medium, 3 – severe clinical changes) by two independent persons. Score is significantly higher in all groups which were not been treated with BPC 157. Daily diuresis has been measured in metabolic cages, polyuria is significantly higher and starts earlier in groups which are not treated with BPC 157, and oliguric phase is not noted in BPC 157 treated rats. Serum levels of creatinine, urea, potassium, sodium, calcium and magnesium after venepunction from vena cava have been analysed. Significantly higher levels of urea and creatinine and lower levels of sodium are noted in groups that are not treated with BPC 157, and so far no significant difference in serum levels of potassium, magnesium and calcium between groups is noted. Rats are sacrificed with double dose of ketamine and kidneys without perirenal tissue are taken, relative mass (RM) of kidney has been measured and results show higher RM in all groups that are not treated with BPC 157 which is probably caused by interstitial oedema. Finally, kidney samples will be patohistologically analyzed and after that statistical analysis of the results will be done.

Conclusion: so far we have proven that pentadecapeptide BPC 157 has a nephroprotective effect but further researching is necessary.

Keywords: pentadecapeptide BPC 157, aminoglycoside nephrotoxicity, rats

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POSTER PRESENTATION

THE EFFECTS OF PENTADECAPETIDE BPC 157 ON THE OPENING OF PREEXISTING COLLATERALS AND ON HEALING OF DUODENAL MUCOSA AFTER LIGATION OF ANTERIOR PANCREATICODUODENAL VEIN IN RATS

Introduction: There is a certain number of inactive blood vessels in various tissues, which do not participate actively in blood distribution and are activated in case of blood stasis. We hypothesize that pentadecapeptide 157 might induce activation of those blood vessels, increase their number and speed up their activation.

Materials and methods: The experiment will be conducted on female Wistar albino rats. Ligation of anterior pancreaticoduodenal vein is performed in both control and BPC treated animals at 1 cm below duodenum. BPC 157 will be applied locally on duodenum. We will observe the speed of opening and number of collateral blood vessels 5 minutes, 30 minutes and 24 hours after ligation. Length of observed duodenal segment is determined from the pylorus to the last congested blood vessel. Collaterals will be counted between the congested main blood vessels using a microcamera and the number of those vessels will then be compared. After recording, animals will be sacrificed, their duodenum extirpated and sent to pathological analysis.

Results: Duodenum of animals were recorded under microcamera (40x magnification) during 5 minutes. Video image was transformed in digital picture in order to count collaterals. We observed 5 control group animals and 5 animals treated with BPC 157 and counted collateral blood vessels on their duodenum 5 minutes after ligation of anterior pancreaticoduodenal vein. Average number of blood vessels in control animals was 108 while in treated animals it was 217. There were two times more counted collateral blood vessels in treated animals, oppose to control ones. Standard deviation was 11.1 for control groups and 21 for treated groups, with coefficient of variation of 10.2 for control groups and 9.9 for BPC157 treated ones. In 60% of control animals we observed duodenal ulcers while we observed the same in 40% of treated animals.

Conclusion: Results might indicate positive effect of BPC 157 in healing of congested duodenal mucosa and show larger number of activated blood vessels in BPC157 treated animals, opposed to control ones. These results must be expanded on larger number of animals and subjected to more detailed statistic analysis in order to have a definitive conclusion.

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POSTER PRESENTATION

PENTADECAPEPTIDE BPC 157 AND WOUND HEALING AFTER HIND LIMB ISCHEMIA

Introduction: Recent studies have shown the positive effect of pentadecapeptide BPC 157 on lesions of various organic systems and its effect on the NO system. No study on the effect of the pentadecapeptide BPC 157 on hind limb wound healing in ischemic conditions has been published so far. We have, therefore, analysed its effect produced by permanent ligation of common iliac artery.

Aim: The aim of this study is to prove that the administration of pentadecapeptide BCP 157 results in quick wound healing in circumstances of induced ischemia of the hind limbs, during the permanent ligation of the common iliac artery, and that this phenomenon is mediated by the action of the NO system.

Materials and methods: The experiment was performed on 255 male Wistar Albino rats. The animals underwent surgery aimed at the ligation of common iliac artery and hind limb wound under deep anaesthesia. The animals were randomly divided into 11 groups and were treated with saline, BPC 157, L arginine, L NAME and cream (neutral and cream with BPC 157). The animals were sacrificed and assessed at the end of each single experimental period (24 hrs, 3,7,14 and 21 days). The assessed parameters were: macroscopic, functional and microscopic analysis of the hind limb skin wound.

Results: Contrary to controls, animals treated with BPC 157 showed better and faster healing of hind limb wounds, better wound contracture, less healing complications and better vascularization after common iliac artery ligation. Also, despite severe induced ischaemia, the status of hind limb ischaemia was promptly reduced and almost completely gone within 24 hrs.

Discussion and Conclusion: The combination of the BPC 157 and L-arginine strengthened the otherwise mild positive effect of L-arginine. The negative effect of L-NAME (difficult and slower wound healing in comparison to the control group) was annulled by the effect of L-arginine and vice versa (L-NAME+L-arginine reached control level), whereas the administration of BPC 157, besides annulling the negative effect of L-NAME, not only brought its damaging effect to regression but also resulted in a positive effect in the wound healing process (L-NAME +BPC 157, L-NAME+L-arginine+BPC 157 resulted in a reduction below control level). A similar effect was partly noticed in the administration of L-arginine, whereas the opposite was achieved by administering L-NAME. All mentioned positive effects of the BPC 157 were achieved regardless of the dose and administration modality. Our study furthermore shows that the NO system undoubtedly contributes to the healing of ischemic wounds of the skin and that a positive effect of the BPC 157 in the healing process is mediated by the action of the NO system.

Keywords: pentadecapeptide BPC 157, NO system, wound healing, ischemia, hind limb, L-arginine, L-NAME