

CAPSAICIN-SENSITIVE AFFERENTATION AND HUMAN GASTROINTESTINAL TRACT

Doctoral (Ph.D.) thesis

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“Optimalisation of drug”

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Pécs, 2014.

Abbreviations

ASA	acetylsalicylic acid
BAO	basal acid output
CGRP	calcitonin gene-related peptide
d	day
ED₅₀	50 % effective dose
GI	gastrointestinal
GTPD	gastric transmucosal potential difference
h	hour
H. pylori	Helicobacter pylori
IND	indomethacin
i.g.	intragastrically
i.v.	intravenous
min	minute
PPI	proton pump inhibitor
s.c.	subcutaneous
SP	substance P
SS	somatostatin
TRPV1	transient receptor potential vanilloid 1
[¹³C] UBT	[¹³ C] urea breath test

1. INTRODUCTION

Capsaicinoids (capsaicin, dihydrocapsaicin, nordihydrocapsaicin and other capsaicinoids) are the active ingredients of red pepper and paprika, and these have been widely applying in the every day culinary.

It was an important discovery that the capsaicin(oids) specifically modifies (modify) the function of certain nerves, later named to capsaicin-sensitive afferent nerves (Jancsó et al., 1967; 1968; 1970). Capsaicin(oids) activates (activate) the capsaicin (vanilloid) receptor expressed a subgroup of primary afferent nociceptive neurons (Szolcsányi, 2004). The capsaicin receptor has been cloned (Caterina et al., 1997) and turned out to be a cation channel. It is gated besides capsaicin and other capsaicinoids (some vanillioids) by low pH, noxious heat and various pains-producing endogenous and exogenous chemicals. Thus, these sensory nerve endings equipped with these ion channels are prone to be stimulated in gastric mucosa.

The actions of capsaicin on the capsaicin-sensitive afferent nerves have been found to be dose-dependent. Szolcsányi (1984) observed four different stages of capsaicin action on afferent nerves (depending on the dose and duration of the exposure of the compound), which include: (a) excitation (stage 1); (b) sensory blocking effect (stage 2); (c) long-term selective neurotoxin impairment (stage 3); and (d) irreversible cell destruction (stage 4). The stages 1 and 2 are reversible, whereas stages 3 and 4 are irreversible compound-induced actions on the capsaicin-sensitive afferent nerve. These stages of capsaicin actions can be detected in the gastrointestinal tract.

Szolcsányi and Barthó (1981) have been observed firstly in the World that the capsaicin(oids) given in small (excitatory) doses prevents (prevent) the gastric mucosal damage, meanwhile it (they) enhances (enhance) the gastric mucosal damage when it (they) was (were) given in higher doses. After this time, professor Peter Holzer (Graz, Austria) carried out very deep and wide scale of animal observations with capsaicin(oids) and he respected the results of Szolcsányi and Barthó.

The neurotransmitters [substance P (SP), calcitonin-gene related peptide (CGRP), somatostatin (SS), etc.,] are released by the administration of small doses of capsaicin (from ng/kg to µg/kg body weight) from these endings and these mediators are responsible for the physiological effects of capsaicin in the gastrointestinal (GI) tract.

The vagal nerve has a key-role in the development of gastrointestinal mucosal damage and prevention. The key-role of the vagal nerve has been emphasized dominantly in the

aggressive processes to gastrointestinal mucosa (such as in peptic ulcer disease, gastric mucosal damage, etc.) as evidenced from gastrointestinal investigations in animal models and as well as in human clinical practice. Thus, “chemical” and “surgical” vagotomy was widely used in the treatment of patients with peptic ulcer disease over the years up to mid-1970s. The primary aims of this therapy were then to decrease the activity of vagal nerve at the level of efferent vagal fibers.

The application of capsaicin in the animal experiments was used as a specific tool to investigate those primary afferent nociceptive neurons involved in the different physiological and pathological processes.

Our work team also has been contributed in the gastrointestinal capsaicin research from the 1980’s in animal models and from 1997 in human healthy subjects. The new and interesting results obtained with capsaicin application in animal experiments offered an excellent tool to approach the different events of human gastrointestinal physiology, pathology and pharmacology.

The actions of capsaicin were studied on the gastric basal acid output (BAO), changes in the “parietal” and “non- parietal” components of gastric secretion, gastric emptying, gastric transmucosal potential difference (without and with intragastric application of ethanol), gastric microbleedings (without application of any drug, and with application of indomethacin) in healthy human beings.

The stimulatory doses of capsaicin were obtained in range from 200 to 1200 µg orally given, and the 50% of the effective dose (ED₅₀) value is equal to 400 µg orally.

These studies were permitted by the Permission of Regional Ethical Committee (Pécs University, Hungary).

It has been proven that the capsaicin produced dose-dependent manner action in healthy human subjects: a. decrease of gastric basal acid output (BAO); b. increase of gastric transmucosal potential difference (GTPD), c. prevention of ethanol-induced decrease of gastric transmucosal potential difference); d. prevention of indomethacin-induced increased gastric microbleedings. and e. the 400 µg intragastrically given capsaicin (ED₅₀) decreased the “parietal component” in association with the increase of “non-parietal” component of gastric secretion. The results of these observations were published by our work-team).

These human observations with capsaicin were well planned, carried out, and under controlled conditions. However, we wanted to study the actions of capsaicin in patients with different natural gastrointestinal disorders.

We have to respect the following main aspects of the different GI disorders in patients:

- a. We have no correct information on the onset of different diseases;
- b. No informations were found in the world literature on the participation of capsaicin-sensitive afferent nerves in different targets of patients with different gastrointestinal disorders;
- c. The patterns of patients appearing at the special consultations and at hospital services are independent from the researcher persons;
- d. The ethical laws are well known in patients' health services and we have to ask further special ethical permission from the Regional Ethical Committee of Pécs University (Hungary) for these studies;
- e. The limitation possibilities are present in our hand to obtain different tissue samples for studies;
- f. The authors have a significant clinical limitation to obtain so-called "homogenous" group(s)" of patients for doing studies;
- g. Only the specific immunohistochemical methods can be used in approach the potential participation and/or the role of capsaicin-sensitive afferent nerves in these disorders (under these clinical conditions).

Different and specific immunohistochemical examinations were carried out in different organs (stomach, duodenum, colon) of patients with different GI disorders (erosion, ulcer, chronic gastritis, polyps, adenocarcinoma of stomach, inflammatory bowel diseases, polyps, severe dysplasia, adenocarcinoma of colon). These patients appeared at our health services in the time period from 2003-2010.

Systematic observations were carried out in patients with chronic gastritis. The chronic gastritis can be produced by a wide scale of causative factors (chemicals, drugs, viral and bacterial infections, immune mechanisms, bile and acid refluxes, etc.). Our attention was focused to the potential role(s) of capsaicin-sensitive afferentation in the development of H. pylori positive and negative chronic gastritis, and on the other hand, in the healing of Helicobacter pylori positive gastritis on dependence of eradication treatment in patients.

Finally we collected healthy subjects to study the effect of capsaicin (in $ED_{50}=400 \mu\text{g}$) on the glucose absorption from the small intestine, because the capsaicin-produced vasodilatation in the small intestinal mucosa has been proven in animal observations. The glucose absorption represents an active transport process [including its absorption, different hormonal (insulin, glucagon) regulatory mechanisms after

absorption and synthesis of glycogen and mobilization in the liver. These mechanisms can be separated very well in time periods after glucose loading test in the healthy subjects].

2. AIMS OF THE PRESENT STUDIES

2.1. To study the possible presence and distribution of capsaicin receptor (TRPV1) and the capsaicin-sensitive afferent nerves liberated neurotransmitters (CGRP, SP) in the GI mucosal tissue samples in patients with different GI disorders (independently on the different stages of different diseases and of their medical treatments);

2.2. To compare the changes in the presence and in distribution of capsaicin receptor (TRPV1) and CGRP, SP in the gastric mucosa of H. pylori positive or negative chronic gastritis in patients. We had no exact knowledge on the origin of chronic gastritis, however, we examined by different [¹³C] urea breath test, rapid urease test, Warthin-Starry staining and special histological methods] methods the presence or absence a H. pylori infection in the gastric mucosa of examined patients;

2.3. To study and to compare the efficacy of eradication treatment vs. changes in the TRPV1 and CGRP, SP by specific immunohistochemical methods in the gastric mucosa in patients with H. pylori positive gastritis before and after (during the time of medical treatment) eradication treatment;

2.4. To evaluate the action of capsaicin(oids) on the glucose absorption of glucose from small intestine and to analyze the hormonal regulatory mechanisms of serum level of glucose after orally given 75 g glucose without and with capsaicin (ED₅₀=400 µg) application in human healthy subjects.

3. MATERIALS AND METHODS.

3.1. Patients and human healthy subjects.

These observations were carried out in 250 subjects (196 patients with different gastrointestinal disorders and 54 human healthy subjects).

The human examinations were permitted by the Regional Ethical Committee of University Pécs, Hungary. Written informed consent was obtained from all participants.

3.1.1. Immunohistochemical distribution of vanilloid receptor, calcitonin-gene-related peptide and substance P in gastrointestinal mucosa of patients with different gastrointestinal disorders

Patients (127 in total, 68 males and 59 females) suffering different gastric and colon diseases were included into this study. The age of patients was 21–84 years. The patients had superficial gastritis (51), erosive gastritis (5), gastric ulcer (4), gastric polyps (6), gastric adenocarcinoma (4), inflammatory bowel disease (17), polyps and hyperplasia (17), polyps with dysplasia (17) and colon adenocarcinoma (6).

3.1.2 Participation of capsaicin-sensitive afferent nerves in the gastric mucosa of patients with *Helicobacter pylori* positive or negative chronic gastritis

Fifty one patients suffered from chronic gastritis with or without *H. pylori* infection (21 *H. pylori* positive, 30 *H. pylori* negative) were admitted in this study. Twenty peoples (10 males and 10 females) with functional dyspepsia were taken as healthy controls. The age of patients was 39 to 68 years.

3.1.3. Changes in the capsaicin-sensitive afferentation (TRPV1, CGRP, SP) in the gastric mucosa of patients with *H. pylori* positive gastritis before and after eradication treatment.

These observations were carried out in 38 persons, including 20 healthy subjects (10 males and 10 females) and 18 patients (6 males, 12 females) with *H. pylori* positive gastritis. The age of patients was 39 to 68 years (mean = 56.4 years. Twenty peoples with functional dyspepsia were taken as healthy controls (age of patients was 41-67 years; mean = 52.1 years).

3.1.4. Capsaicin and glucose absorption and utilization in healthy human subjects.

Fourteen healthy human subjects (aged 45 ± 5 , mean \pm S.E.M.) participated in this self-controlled study.

3.2. Investigation methods.

All the participants of the immunohistochemical observations were admitted in the First Department of Medicine, Medical and Health Centre, University of Pécs or in the Department of Medicine and Gastroenterology, Markusovszky Teaching Hospital, Szombathely (Hungary) for medical (physical, laboratory, ultrasonography, endoscopic and histological) examinations.

The mucosal biopsies were collected from the pathognomonic areas of the gastric/large bowel mucosa during endoscopic procedure.

The GI tissue samples were analyzed in the Department of Pathology by an independent histopathologist, and classified into different groups according to classical pathological diagnosis (Sydney's system).

3.2.1. Detection of *H. pylori* infection

The *H. pylori* infection was detected by using [¹³C] urea breath test ([¹³C] UBT), rapid urease test, Warthin-Starry silver staining and specific histological examinations. The gastric tissue samples were classified into different groups of chronic gastritis according to the Sydney's System.

3.2.2. *H. pylori* eradication protocol

H. pylori positive patients underwent 7 days long eradication treatment with the combination of double dose PPI (pantoprazole 2 × 40 mg/d), amoxicillin (1000 mg twice daily) and clarithromycin (500 mg twice daily) according to current European guidelines. After this 1 week combination therapy, patients continued to take a normal dose of PPI for another week. The time period between the first and control gastroscopy was 6 weeks. Similar gastric biopsies were taken from the corpus and antrum of healthy subjects.) A questionnaire was used to determine the patients' symptoms before and after the eradication therapy.

3.2.3. Immunohistological methods

The immunohistological studies were carried out on formalin fixed, paraffin embedded tissue samples of gastric mucosa using the peroxidase-labeled polymer method (Lab Vision Co., Fremont, USA). SP was detected by the NC1/34 HL rat monoclonal antibody, the TRVP1 and CGRP were labeled using polyclonal rabbit antisera (all from Abcam Ltd., Cambridge, UK). The immunohistochemical analysis was assessed by light microscopy (Olympus).

The immunohistochemical examinations were performed in the Histopathology Ltd., Pécs (Hungary). The TRPV1 and CGRP were detected as positive or negative,

meanwhile the SP immunodistribution was characterized by using a „SP-index”. This index was calculated by counting immunopositive spots in at least five high magnification fields. In fields without immunostaining, the score was zero, fields containing only one positive spots, the score was one and fields with two or more stained elements were scored two. The total score in one specimen was divided by the number of the scanned field to obtain the SP-index. Based on these results, biopsies were classed into three categories; weak, medium and strong (Table 1.).

Table 1. Semiquantitative quantition of immunohistochemical staining of SP in the gastric mucosa of healthy subjects and of patients with chronic gastritis.

SP evaluation	SP-index
Weak	$<0,5$
Medium	$0,5 \leq \text{and} <1$
Strong	$1 \leq$

3.2.3. Glucose loading test and the measured parameters

Each healthy subject received 75 g glucose orally (dissolved in 100 ml of water), without and with capsaicin (400 µg orally given, Sigma-Aldrich, Budapest, Hungary). The plasma levels of glucose (Boehringer, Germany), insulin (µIU/mL) (Biochem Immunsystem), C-peptide (ng/mL) and glucagon (pg/mL) (Byk-Sangtect Diagnostic GmbH) were measured in every 15 min for 4 hour.

3.2.4. Statistical analysis of the results

Immunodistribution of TRPV1 and CGRP were statistically evaluated by χ^2 -probe, meanwhile the SP results were semi quantitating evaluated by Mann-Whitney’s U test. The paired Student T-test was applied for statistical analysis during glucose loading test with or without administration of capsaicin. The results were taken to be significant, if P values were $\leq 0,05$.

4. RESULTS

4.1. Presence and distribution of TRPV1 and CGRP, SP in the GI mucosa with different gastrointestinal disorders.

The immunostaining of TRPV1 was detected as fine granular cytoplasmic immunosigns in the epithelial cells at gastric and colon mucosa. In patients with different large bowel diseases, immunosignal of TRPV1 could be detected intracytoplasmatic as “spot-like pattern”.

The characteristic immunmdistribution of CGRP was fine granular cytoplasmic positivity in the epithelial cells at GI mucosa.

The immunomorphology of SP was detected as granular small spot-like signals along the mucosal blood vessels at gastric mucosa of healthy subjects and of patients with chronic gastritis with or without presence of *H. pylori* infection.

The immunodistributions of TRPV1, SP and CGRP in patients with different gastric disorders are summarized by Table 2.

Table 2. Summary of immunohistochemical changes in the gastric mucosa in patients with different gastric disorders. The numbers between the parentheses represent the number of the patients.

	Control	Chronic gastritis	Gastric erosion	Peptic ulcer	Gastric polyps	Adenoc. ventriculi
TRPV1						
positive	20% (4)	69% (35)	80% (4)	50% (2)	66.7% (4)	66.7% (2)
negative	80% (16)	31% (16)	20% (1)	50% (2)	33.3% (2)	33.3% (1)
CGRP						
positive	30% (6)	59% (30)	60% (3)	50% (2)	66.7% (4)	100% (4)
negative	70% (14)	41% (21)	40% (2)	50% (2)	33.3% (2)	0% (0)
SP						
weak	75% (15)	59% (29)	60 % (3)	50% (2)	100% (6)	80% (3)
moderate	0% (0)	14% (7)	0% (0)	0% (0)	0% (0)	20% (1)
strong	25% (5)	27% (13)	40% (2)	50% (2)	0% (0)	0% (0)

Table 3. summarizes the immunodistribution of TRPV1, CGRP and SP in patients with different large bowel disorder.

Table 3. Summary of TRPV1, CGRP, SP distribution in the colon mucosa of patients with different large bowel diseases. The numbers between the parentheses represent the number of the patients.

	Inflammatory bowel diseases	Polyps with moderate dysplasia	Polyps with severe dysplasia	Colon adenocarcinoma
TRPV1				
Negative	29.4% (5)	70.6% (12)	17.6% (3)	16.7% (1)
Positive	59.8% (10)	0% (0)	5.9% (1)	0% (0)
“spot-like pattern”	11.8% (2)	29.4% (5)	76.5% (13)	83.3% (5)
CGRP				
Negative	40.2 % (7)	82.4 % (14)	94.1 % (16)	16.7 % (1)
Positive	59.8 % (10)	12.6 % (3)	5.9 % (1)	83.3 % (5)
SP				
Weak	5.9% (1)	70.6% (12)	82.4% (14)	66.6% (4)
Moderate	17.6% (3)	11.8% (2)	5.9% (1)	16.7% (1)
Strong	76.5% (13)	17.6% (3)	11.8% (2)	16.7% (1)

All three examined immunohistochemical changes are present in inflammation. TRPV1 is also present in dysplasia and tumors, and CGRP in tumors; however, SP is not typically present in polyps with moderate and severe dysplasia and tumors of the colon (Table 4).

Table 4. Summary of the immunohistochemical changes in the colon mucosa of patients with different large bowel disorders.

	TRPV1	CGRP	SP
Inflammatory bowel disease	+	+	+
Polyps with moderate dysplasia	-	-	-
Polyps with severe dysplasia	+	-	-
Colon adenocarcinoma	+	+	-

4.2. Presence and distribution of TRPV1 and CGRP, SP in the gastric mucosa of patients with Helicobacter pylori positive and negative chronic gastritis

The TRPV1 positive cases of chronic gastritis were significantly higher ($P < 0.01$) extent than in controls meanwhile no significant difference was detected in the immunomorphology of TRPV1 between the patients with H. pylori negative or positive chronic gastritis.

In the immunohistological distribution of CGRP, significant changes could be observed between the healthy and H. pylori negative chronic gastritis ($P < 0.01$) and no significant difference was found in the two types (H. pylori negative or positive) of chronic gastritis. Although the count of the positive tissue samples increased in H. pylori positive gastritis, it did not reach significantly different level compared to healthy controls.

No significantly change could be observed in the amount of weak and strong SP-score between the healthy subjects and the patients with chronic gastritis meanwhile the medium cases of SP immunohistological samples appeared in chronic H. pylori negative or positive gastritis.

The summary of the immunohistochemical results is demonstrated by Figure 1. and Table 5.

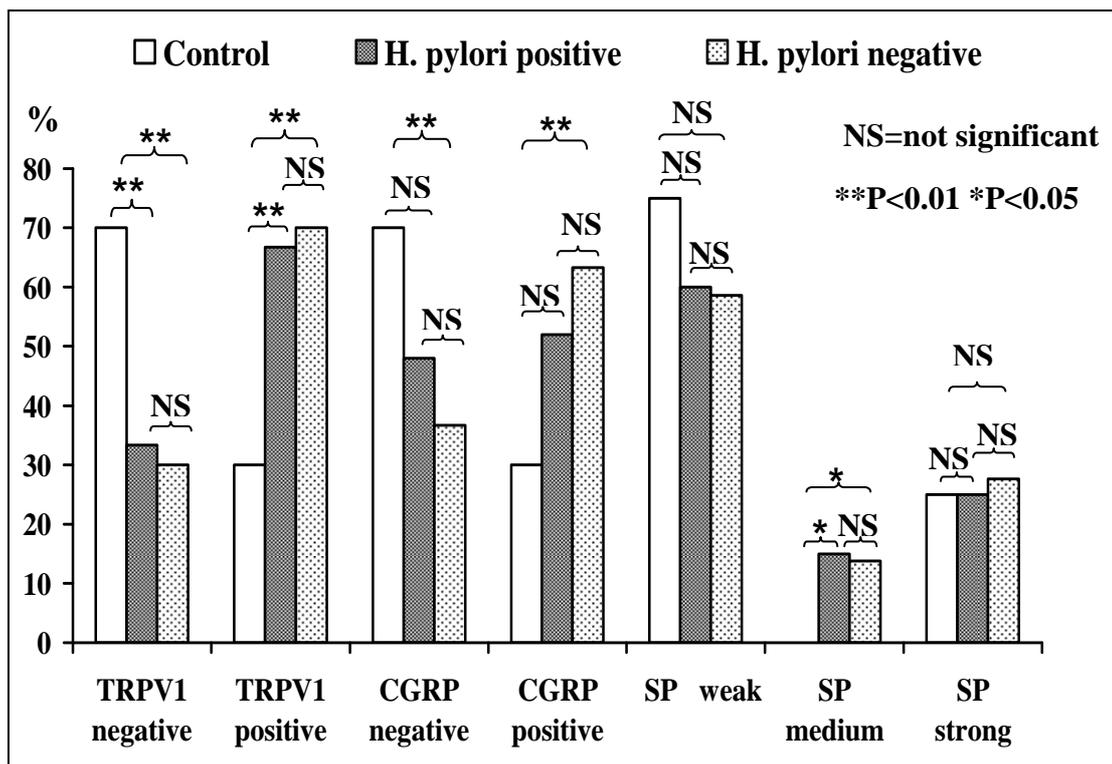


Figure 1. The results of immunodistribution of TRPV1, CGRP and SP in the gastric mucosa of patients suffered from H. pylori positive or negative chronic gastritis and in healthy subjects.

Table 5. Summary of immunodistribution of TRVP1, CGRP and SP in the gastric mucosa of patients suffered from H. pylori positive or negative chronic gastritis and in healthy subjects. The numbers between the parentheses represent the number of the patients.

	TRPV 1 positive	TRPV 1 negative	CGRP positive	CGRP negative	SP weak	SP medium	SP strong
Control	20 % (4)	80 % (16)	30 % (6)	70 % (14)	75 % (15)	0 % (0)	25 % (5)
H. pylori positive	66.7 % (14)	33.3 % (7)	52 % (11)	48 % (10)	60 % (12)	15 % (3)	25 % (5)
H. pylori negative	70 % (21)	30 % (9)	63.3 % (19)	36.7 % (11)	58.6 % (17)	13,8 % (4)	27.6 % (8)

4.3. The efficacy of eradication treatment and the changes in the immunodistribution of TRPV, CGRP and SP in the gastric mucosa in patients with H. pylori positive gastritis before and after (during the time of medical treatment) eradication treatment;

Before H. pylori eradication, the symptoms of patients with H. pylori positive chronic gastritis were unspecific: epigastric pain (14/18, 77%), heartburn (13/18, 72%), nausea/vomiting (9/18, 50%) abdominal expansion (9/18, 50%), constipation (6/18, 33%).

Moderate and severe activity of inflammation could be observed in the gastric biopsies of patients with *H. pylori* positive chronic gastritis before eradication treatment.

The *H. pylori* eradication therapy was successful in 16 of 18 patients (89%).

After eradication, symptoms were found to remain moderate in seven patients (7/18, 39%) and 11 patients (11/18, 61%) had no complaints after treatment.

Histologically healthy gastric mucosa could be detected only in 4 cases of the control biopsies and in 14 cases the appearance of chronic gastritis was moderated.

Fine cytoplasmic TRVP1 positivity was detected in 37% (7/20) of healthy subjects, whereas *H. pylori* positive gastritis patients were 89% (16/18, $P < 0.001$) positive before and 72% (13/18 $P < 0.03$) positive after eradication therapy.

Immunohistochemistry for CGRP was positive in 100% (18/18, $P < 0.001$) of patients both before and after eradication (18/18, $P < 0.001$). In the mucosa of healthy individuals this staining was found to be positive in 40% (8/20) of controls.

SP was detected as small granular spot-like signals localized along the mucosal blood vessels. The SP immune-staining was positive in 25% (5/20) of control persons, and in 5.5% (1/18, $P > 0.05$) before and 0% (0/18, $P > 0.05$) after eradication in the gastric mucosa.

The results of the immunohistological examinations for TRVP1 and mediators (CGRP and SP) are summarized in Table 6. and Figure 2. Interestingly, when compared to normal mucosa, the presence of SP decreased in the mucosa obtained from patients with *H. pylori* positive chronic gastritis before and after eradication treatment (Figure 2.).

Table 6. Summary of the presence of capsaicin receptor, CGRP and SP in the gastric mucosa of patients with chronic *H. pylori* positive gastritis, before and after eradication therapy, obtained by immunohistochemistry. The numbers between the parentheses represent the number of the patients.

	TRPV1		CGRP		Substance P	
	Positive	Negative	Positive	Negative	Positive	Negative
Before eradication (n=18)	88.89 % (16)	11.11 % (2)	100 % (18)	0 % (0)	5.56 % (1)	94.44 % (17)
After eradication (n=18)	72.22% (13)	17.78% (5)	100 % (18)	0 % (0)	0 % (0)	100 % (18)
Control group (n=20)	35 % (7)	65 % (13)	40 % (8)	60 % (12)	25 % (5)	75 % (15)

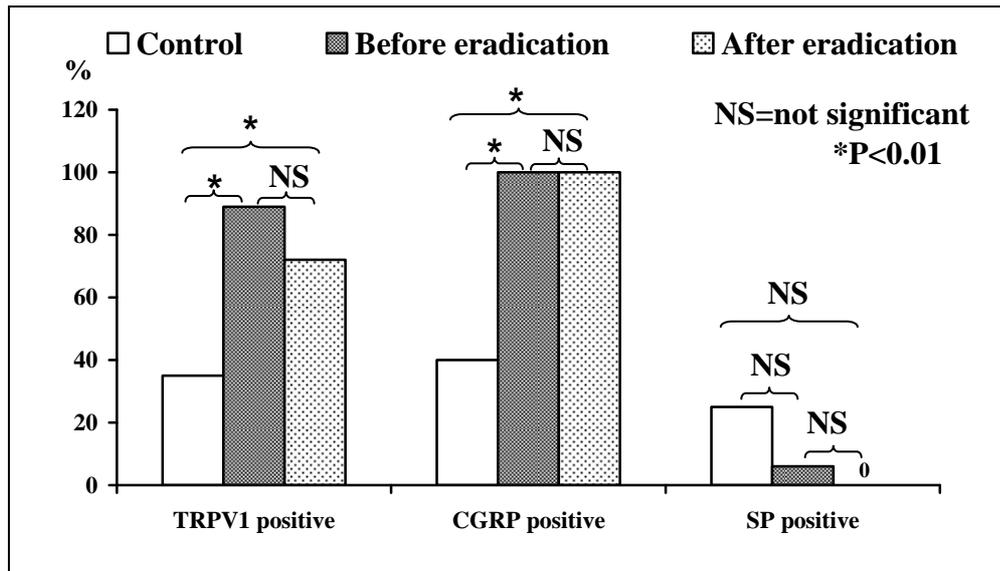


Figure 2. Changes in the immunodistribution of capsaicin receptor (TRPV1), CGRP and SP in patients with *H. pylori* positive chronic gastritis before and after eradication.

4.4. “Capsaicin and glucose absorption and utilization in healthy human subjects”

The plasma levels of glucose increased significantly 30 to 150 min and the plasma level of glucagon increased from 90 to 180 min after capsaicin administration in human healthy subjects given 75 g glucose orally. The plasma levels of insulin and C peptide increased from 75 to 165 min after glucose administration; however, levels did not differ significantly without or with capsaicin (400 μ g orally) (Fig. 3).

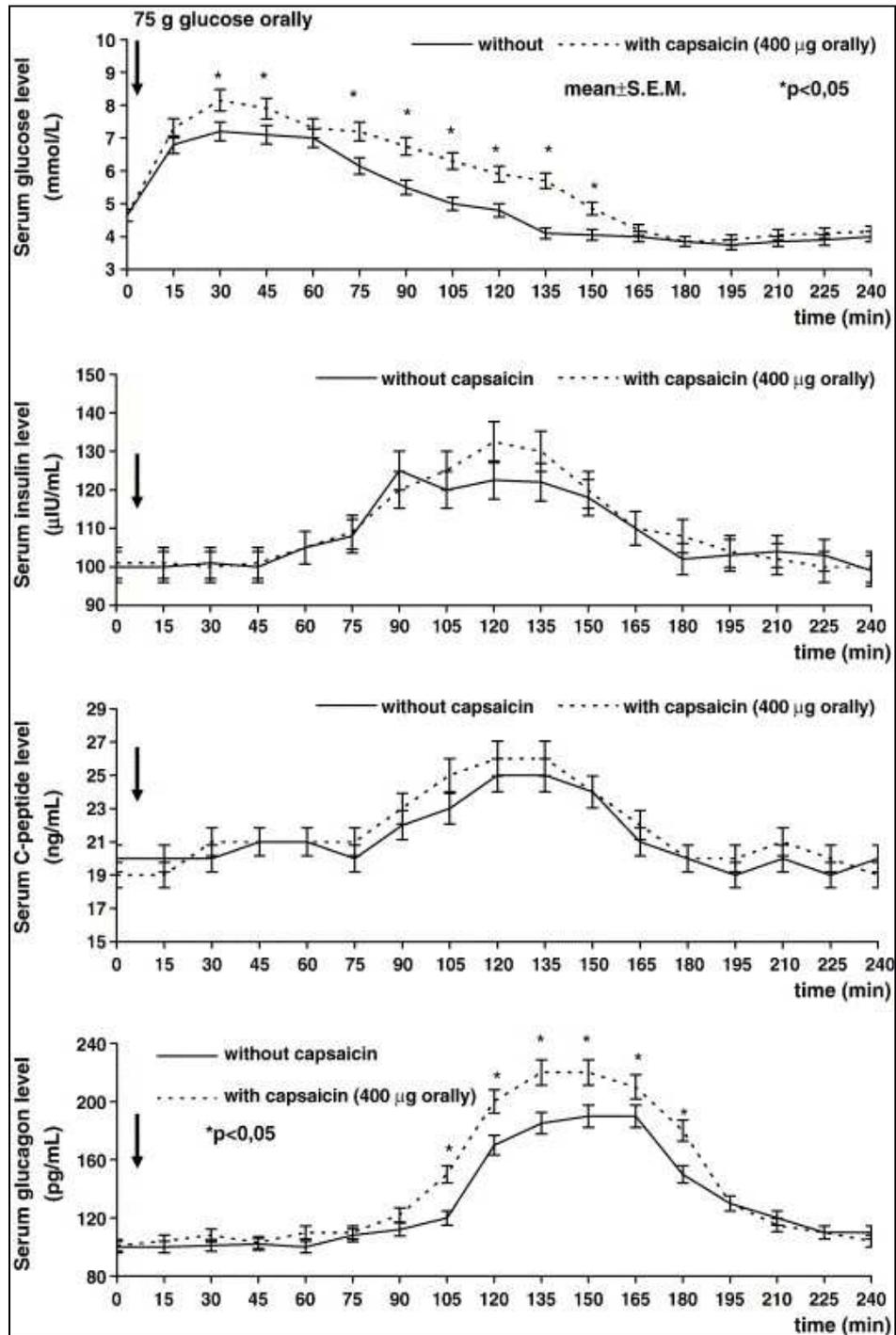


Figure 3. Changes in plasma levels of glucose, insulin, C-peptide and glucagon after oral administration of glucose (75 g in 100 ml water) in 14 healthy human subjects. Capsaicin (400 µg) was orally given in gelatin capsule (Hunгарopharma, Budapest, Hungary). The plasma levels of glucose, insulin, C-peptide and glucagon were measured every 15 min for 4 h. The results are expressed as means ± S.E.M.

5. DISCUSSION

Our work team earlier published the results of capsaicin research in the animal experiments (Mózsik et al., 1997), and later in human healthy subjects (Mózsik et al., 2005; 2014; Mózsik, 2014).

These examinations were well planned and these were carried out studies in prospective, randomized manner under the conditions of Good Clinical Practice (GPC). The results of these observations clearly proved that capsaicin (given orally in small – excitatory - doses) prevents the ethanol- and indomethacin-induced gastric mucosal damage. It was also important to note that no desensitization developed in two weeks capsaicin (3x400 µg/day orally) treatment.

The presented results in the thesis were the first objective data in this field, consequently there was a difficulty to carried out critically them.

These results indicated well that the capsaicin-sensitive afferentation take place in the development of different gastrointestinal disorders (see 4.1.chapter), however, we did not receive correct possibility to evaluate the harmful or beneficial role of capsaicin receptor function and released neuropeptides (CGRP, SP) in these diseases.

The results of the study with patient suffered from chronic gastritis suggest that the TRPV1 and CGRP are involved in the development of chronic gastritis and this role is not depending on the cause of the disease.

We were surprisingly noted that the “upregulation” of TRPV1 and CGRP unchanged (not decreased) during (before and after) the eradication treatment in *H. pylori* positive chronic gastritis of patients, meanwhile the eradication was practically successful. These results offered us to suggest that the capsaicin-sensitive afferentation-induced gastric mucosal defensive action is independent from the eradication treatment in patients with chronic gastritis. This recognition offers a new possibility to introduce a capsaicin-containing compound in the medical treatment of patients with chronic gastritis.

Finally the glucose loading test examinations indicated clearly that the glucose absorption enhanced from the small intestine in association with increased glucagon release by the application of capsaicin(oids) in healthy human subjects. These results call our attention to conclude that the capsaicin(oids) participates (participate) in carbohydrate metabolism.

The possible role of capsaicin action(s) represents (represent) a special difficulty in the capsaicin research in these studies of patients with different gastrointestinal disorders.

6. NEW RESULTS

1. The TRPV1 and SP and CGRP could be detected by immunohistochemical method in tissue samples of patients with different GI disorders. It means that the capsaicin-sensitive afferent nerves and released neuropeptides by them take part in the development of different human GI disorders.
2. The immunohistochemical distribution of TRPV1, CGRP and SP differs in the upper (gastric) and distal (colon) parts of the gastrointestinal tract (inflammation, hyperplasia, dysplasia, ulcer, polyps and cancers).
3. The involvement of capsaicin-sensitive afferent nerves in human chronic gastritis does not depend on the presence of *H. pylori* infection,
4. The capsaicin-sensitive afferentation has a permanent defensive role in the human gastric mucosa against injury produced by different noxious agents.
5. The increased activation (compared with histological healthy persons) of capsaicin-sensitive afferent nerves (by immunohistochemical distribution of TRPV1, CGRP and SP) after successfully *H. pylori* eradication therapy suggests that these nerves are involved in the healing mechanism of the gastric mucosa (that is a new capsaicin-sensitive afferent neural defensive pathway or mechanism in the treatment of *H. pylori*-induced chronic gastritis). This is an internationally new observation in the field of treatment of chronic gastritis in patients.
6. The capsaicin-sensitive afferent nerves have a key role in glucose utilization (increased glucagon release) by tissues in healthy human subjects, indicating the participation of capsaicin action in the human carbohydrate metabolism.

7. PERSPECTIVES IN THE CAPSAICIN-CONTAINING NEW DRUG PRODUCTION IN THE HUMAN THERAPY.

Because the chronic gastric mucosal damage can be induced by different agents (especially by drugs and other causative agents such as *H. pylori*) and the direct orally application of capsaicin prevents the IND-induced mucosal damage and the increased activity of capsaicin-sensitive afferentation measured by immunohistochemical methods in *H. pylori* positive and negative chronic gastritis (which differs from the classical eradication treatment), these results together suggest the existence of the capsaicin-sensitive afferent dependent neural gastro protective mechanism and it suggested that the capsaicin alone or in different combination can be used in the human medical therapy.

The results - as the gastroprotective effect of capsaicin - can be used in the treatment of *H. pylori* induced chronic gastritis, and the capsaicin (given in small doses) is able to prevent the NSAIDs-induced gastrointestinal mucosal damage - suggest that capsaicin might be able to prevent the gastric side effects of NSAIDs used as pain killers, antipyretic drugs or platelet aggregation inhibitors in patients with cardiac diseases, ischemic stroke, chronic joint diseases.

In the last years, the actions of plant origin capsaicin alone or in different combinations (capsaicin + acetylsalicylic acid, capsaicin + diclofenac and capsaicin + naproxen) were done in innovative pharmacological research between the First Department of Medicine, Department of Pharmacology and Pharmacotherapy and Institute of Pharmaceutical Chemistry, Medical and Health Centre, University of Pécs versus the PannonPharma Pharmaceutical Ltd, Pécsvárad. Two from three human phase I. examinations were carried out in human healthy persons (capsaicin + ASA and capsaicin + diclofenac). The results of these finished human phase I. examinations indicated:

1. The capsaicin (orally given in doses of 400 and 800 μg) alone or in combinations could not be detected in the plasma of treated healthy subjects (which suggests that the capsaicin acts primarily locally in the pharmaceutical drug preparation or the pharmaceutical factory used excellent bioadhesive compound during the drug pharmaceutical production);
2. The different doses of capsaicin do not modify the pharmacokinetic parameters of the ASA and diclofenac;
3. Capsaicin(oids) alone has (have) no any effect(s) on the platelet aggregation;

4. The ASA- induced platelet aggregation remained unchanged.
5. The medical application of capsaicin(oids) alone or in some drug combinations (e.g. NSAIDs) offers a new possibility in the medical treatments with different gastrointestinal disorders.

8. ACKNOWLEDGEMENTS

I would like to express my gratefulness to my mentor, Prof. Gyula Mózsik who is not only a great researcher and teacher, but I consider his as a friend of mine.

I am grateful to the Endoscopic Laboratory of the First Department of Medicine, Medical and Health Centre, University of Pécs (Dr. Áron Vincze; Dr. Imre Szabó Dr. József Czimmer and all the assistants) for the support that I have been receiving.

I'd like to warmly thank to Dr. György Szekeres and the co-workers of Histopathology Ltd. (Rita Keszthelyi, Ágnes Meczker, Rebeka Hajós), for the indispensable help provided in the experiments.

I also like to thank the great help of Dr. László Kereskay for the pathological processing and analysis of the tissue samples.

I render thanks to all the staff of the Department of Medicine of Markusovszky Teaching Hospital, Szombathely (especially for Dr. Lilla Lakner and Dr. Zoltán Döbrönte) for their cooperation.

Finally, I would like to thank to my family: to my parents, for giving me a peaceful background and last but not most to my wife, Edit and our daughter Dóra, who completes my life and makes me a better person every day.

Publication related to the dissertation

Published papers

Mózsik, Gy., Peidl, Zs., Szolcsányi, J., **Dömötör, A.**, Hideg, K., Szekeres, Gy., Karád, O., Hunyady, B.: Participation of vanilloid/capsaicin receptors, calcitonin gene-related peptide and substance P in gastric protection of omeprazole and omeprazole-like compounds. *Inflammopharmacology*. 13:139-159. (2005).

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