ORIGINAL PAPERS

Dent. Med. Probl. 2005, **42**, 1, 31–36
ISSN 1644-387X

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Capsaicin Stimulation and Neurogenic Experimentally Induced Inflammation of the Human Labial Mucosa

Stymulacja kapsaicyną zapalenia neurogennego błony śluzowej wargi u człowieka

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Abstract

Background. The neurogenic inflammation depends on the neuromodulator release by peripheral nervous terminations. A series of different studies proved the existence of a neurogenic phlogosis (NP) in different body sites. Only experiences on rat managed to induce NP at the oral mucosa level.
Objectives. Inducing NP at the human mucosa level.
Material and Methods. Two groups of patients (group A: M/F: 8/7; mean: 41.14 ± 12.4; range: 24–60; group B: M/F: 8/7; mean: 41.21 ± 12.39; range: 23–60) were examined in authors' laboratory. The NP was experimentally induced at the lower lip mucosa level after topically applying capsaicin. The vascular consequences were observed through computer videocapillaroscopy techniques (Videocap 100). The lip mucosa of the group A patients was observed in three different time periods: before capsaicin application (t_0), after one minute (t_1), and after 45 minutes (t_2) from capsaicin application. The lip mucosa of the group B patients was observed in four different time periods: before capsaicin application (t_0), after local anaesthetic injected (t_1), after one minute (t_2) and after 45 minutes (t_3) from capsaicin application.
Results. The vascular *in vivo* observation, in the group A, allows to highlight after one minute application only, the axono-reflected vasodilation, the real expression of NP. After 45 minutes, the vascular diameter returned to its initial diameter. In the group B, the vascular *in vivo* study allows to observe no significant modification of the vascular diameter in the four different time periods.
Conclusions. The study highlights that the NP can be induced at the human oral cavity level and such an evidence could be extremely important in the pathogenesis and treatment of the different oral pathologies (**Dent. Med. Probl. 2005, 42, 1, 31–36**).

Key words: neurogenic inflammation, labial mucosa.

Streszczenie

Wprowadzenie. Zapalenie neurogenne zależy od uwalniania neuromodulatorów przez obwodowe zakończenia nerwowe. Wiele różnych badań udowodniło występowanie zapalenia neurogennego (z.n.) różnych części ciała. Zapalenie neurogenne błony śluzowej jamy ustnej wywoływano tylko u szczurów.
Cel pracy. Wywołanie z.n. błony śluzowej u człowieka.
Materiał i metody. Dwie grupy pacjentów (grupa A: K/M: 8/7, średnia wieku 41,14 ± 12,4, zakres 24–60 lat, grupa B: K/M: 8/7, średnia wieku 41,21 ± 12,4, zakres 23–60 lat) zostały zakwalifikowane do badań. Zapalenie neurogenne wywoływano doświadczalnie w błonie śluzowej dolnej wargi po miejscowym zastosowaniu kapsaicyny. Następowe zmiany naczyniowe rejestrowano techniką wideokapilaroskopową (Videocap 100). W grupie A błona śluzowa wargi była oceniana trzykrotnie: przed podaniem kapsaicyny (t_0), po minucie (t_1) i po 45 minutach (t_2) po podaniu kapsaicyny. W grupie B błona śluzowa wargi była oceniana czterokrotnie: przed podaniem kapsaicyny (t_0), po znieczuleniu miejscowym (t_1), po minucie (t_2) oraz po 45 minutach (t_3) po podaniu kapsaicyny.
Wyniki. Obserwacja naczyniowa *in vivo* w grupie A umożliwiła stwierdzanie rozszerzenia naczyń pochodzenia aksonalnego już po minucie po pobudzeniu w postaci z.n. Po 45 minutach średnica naczyń wróciła do wartości wyjściowej. W grupie B badania naczyniowe *in vivo* umożliwiło obserwację nieznaczących zmian średnicy naczyń w czterech różnych przedziałach czasu.

Wnioski. Badanie dowiodło, że zapalenie neurogenne może być wywoływane w jamie ustnej człowieka. Jest to bardzo ważne w patogenezie i leczeniu różnych zmian patologicznych w jamie ustnej (**Dent. Med. Probl. 2005, 42, 1, 31–36**).

Słowa kluczowe: zapalenie neurogenne, błona śluzowa wargi.

Various observations carried out in the course of the years have shown that neuropeptides might play role as inflammation mediators [1–3]. The substances on which attention has been principally focused are: substance P (SP), calcitonin gene-related peptide (CGRP), capsaicin and histamine [4, 5]. Knowledge acquired in this field, however, does not yet permit one to sufficiently clarify the role of these neuropeptides, although it has been confirmed that these substances are found in laboratory animals in the course of experimentally induced neurogenic inflammation [2].

Neurogenic inflammation is a consequence of amyelinic sensitive neuron activation, followed by a neuropeptide release such as SP and CGRP by the peripheral nervous terminations [6].

Inflammatory responses elicited at the tissue level, as a consequence of nervous termination activation mainly consist of: hyperaemia, local oedema and erythema, which mainly develop even beyond the specific stimulation site and therefore can easily be followed by pain [2].

Since when scholars managed to almost definitively correlate such unique inflammatory signs with the peripheral sensitive nervous system function and integrity, the elicited answer is called neurogenic inflammation.

As almost any tissue is innervated by afferent sensitive neurons, a similar mechanism could be involved in the aetiology of many different inflammatory reactions of the oral mucosa [2]. The NP can experimentally be induced by local capsaicin stimulation [7–11]. This is proved by the fact that denervation of the area prevents the development of inflammatory reactions in rats [13].

Capsaicin is a powerful local stimulator which administered in controlled doses can lead to the release of phlogistic neuromodulators and as a consequence to NP [7, 8, 10]. Its long-term administration leads to the small medullar fibre degeneration and reduces held SP by 50% [13]. When increasing the release and inhibiting a further undue influence of any PS, it is possible to lead to a central and peripheral nervous system depletion [2]. A single dose of capsaicin induces pain, inflammation and hypersensitivity. When its application is continued in time, it leads to hyposensitisation, antalgic and anti-inflammatory activity. Any analgesia and mitigant effect possibly depends on the release and depletion of neuropeptides [13–15].

Capsaicin acts as a receptor agonist for the Vanilloids (VR1), a cationic canal, mainly expressed by small sensitive neurons [16, 17]. Axon-reflected vasodilation essentially symmetrically spreads around the nociceptive stimulations mainly extended to the afferent nociceptive stimulation receptor fields [18]. Recent studies on animals proved that a similar inflammatory reaction can be induced by capsaicin through the activation of the different tissue nociceptive fibres [19, 20].

Up to now, a series of experimental studies on the oral mucosa were carried out only on animals. Such a study, on the other hand, aims at assessing the effects of capsaicin application on the human oral mucosa. More precisely, through computer videocapillaroscopy techniques, it aims at assessing the oral micro-circulation *in vivo* before and after the application, checking the possible phlogistic phenomena onset.

Material and Methods

Two groups of patients (group A: M/F: 8/7; mean: 41.14 ± 12.4; range: 24–60; group B: M/F: 8/7; mean: 41.21 ± 12.39; range: 23–60) were examined in authors' laboratory (Tab. 1).

Table 1 Demographic characteristics of patients enrolled in the study

Tabela 1. Charakterystyka pacjentów zakwalifikowanych do badań

	Group A (Grupa A) (n = 15)	Group B (Grupa B) (n = 15)	Significance [a] (Istotność)
M/F ratio (Płeć K/M)	8/7	8/7	
Age (Wiek) (mean ± SD)	41.14 ± 12.4	41.21 ± 12.39	ns
Range (Zakres)	24–60	23–60	

SD – standard deviation.
[a] Differences between group A and B patients were tested by Mann-Whitney U test.
ns – not significant ($p > 0.01$).

SD – odchylenie standardowe.
[a] Do oceny różnic między grupami A i B zastosowano test U Manna-Whitneya.
ns – nieistotne statystycznie ($p > 0.01$).

The subjects were included in the study, if the accurate exam of their medical history and the objective examination of their oral mucous, reported them to be healthy and non smoker. All the subjects gave their written informed consent for processing and use of personal medical data in scientific papers, in accordance with the Italian Law. They were examined by computerised videomicroscopic techniques and related software (Videocap 100). This technique allows a continuous observation of the labial microcirculation.

The optical probe videomicroscope is composed of a main unit, to which an optical probe with video-optical terminal is connected, and by a high resolution colour monitor to view the examined area. The main unit is made of: a cold halogen light source emitted by a 100 W lamp provided with an electronic device which controls light intensity; a processing unit for the high definition video signal (420,000 pixels) provided with a colour calibration device. The probe is equipped with a video-optical terminal containing a high definition video sensor, on which different variable magnification optics from 10× to 1000× can be applied. A technological characteristic of the video-optical terminal is the possibility to focus directly from the handpiece.

Image digitalization allows the analysis of the fundamental parameters of microcirculation (calibre and vessel length), and the calculation of the number of capillaries per mm^2 of the mucosa examined.

The area under investigation for each patient was the inferior labial mucous in the area of the "frenulum". This area was chosen because the macroscopic characteristics of the probe means that this area can be examined without causing discomfort to the patient. The capillaroscopic investigation was carried out with patients in a sitting position; always with the same source of light, at the same room temperature (23°C), in the morning, with the same operator, and repeated twice.

The lip mucosa was observed in three different time periods for the group A patients: before capsaicin application (t_0), after one minute (t_1) and after 45 minutes (t_2) from capsaicin application (0.1%) directly onto the labial mucosa; the lip mucosa of the group B patients was observed in four different time periods: before capsaicin application (t_0), after local anaesthetic injected (t_1), after one minute (t_2) and after 45 minutes (t_3) from capsaicin (0.1%) application.

The following parameters were used:

– visibility of the capillary loops (score from 1 to 4): 1 – simple focusing: within 30 seconds of the start of the examination; 2 – average focusing: over 30 seconds and within 2 minutes; 3 – difficult focusing above 2 minutes; 4 – impossible focusing;

– orientation with respect to the surface (score A or B or AB): A – course of the capillary loops parallel to the surface; B – course of the capillary loops perpendicular to the surface; AB – both parallel and perpendicular;

– capillary tortuosity (score from 0 to 3): 0 – absence of crossing in the capillary loops; 1 – presence of crossing; 2 – presence of more crossings; 3 – complete distortion of the capillary loops;

– calibre of the capillary loops: before and after capsaicin application.

Two independent observers examined all the images. The intraobserver and interobserver variability was assessed with the two observers evaluating twice the same randomly selected images ($p < 0.05$). Data analysis was performed using StatView 5.0.1 (SAS Institute Inc., Cary, NC).

The results obtained for each area examined for every patient were the average of the two observations taken.

Results

Intrasubject variability satisfied the a priori hypothesis of a limited dispersion. For the parametric data, variability ranged between +2% and –2% with respect to the mean value. For the non-parametric data, 1 score point difference at most was observed.

The lip mucosa micro-circulation visibility highlighted a score accounting for 1 in 100% of the patients. The loops were oriented parallel to the surface in 100% of the patients (score A) thus possibly completely assessing the capillary loops. The capillaroscopy, in the group A, highlighted that the vessel diameter after about 1 minute from capsaicin application (t_1), underwent a highly significant increases ($p < 0.0001$ assessed through the ANOVA Test). Extremely long capillary clip-like shaped and mainly volvulated loops protruded into the tissue meshes (Fig. 1).

A further control at 45 minutes (t_2) proved that the vessel diameter and capillary tortuosity returned back to the initial values (Fig. 2a, b). In the group B, the vascular *in vivo* study allowed to observe no significant modification of the vascular diameter and tortuosity in four different time periods ($p > 0.01$ assessed with the ANOVA Test) (Tab. 2).

Discussion

The results of function and morphological studies in rats seem to confirm the possibility of a neurogenic component of inflammatory alterations caused by mechanical, and chemical stimuli

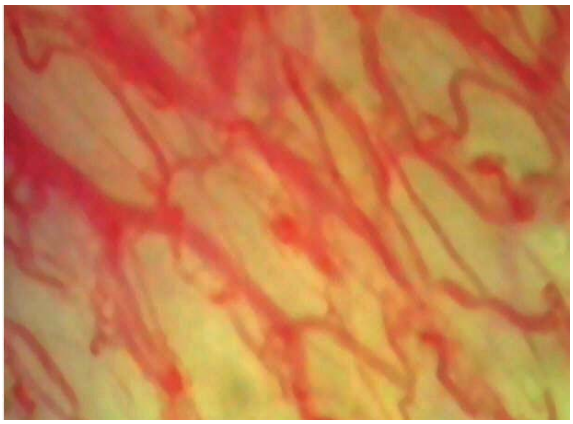
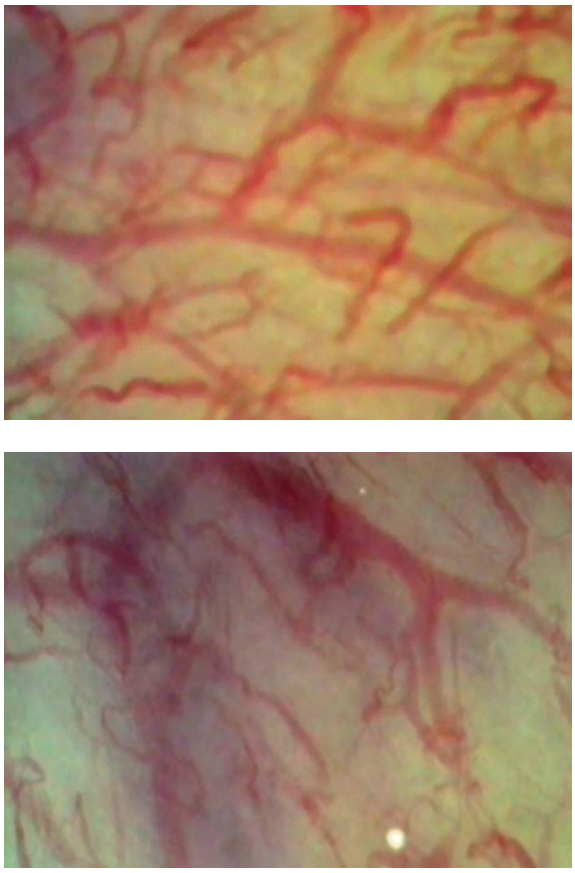


Fig. 1. Group A patient: the vessel diameter after about 1 minute from capsaicin application (t_1), magnification ×200

Ryc. 1. Pacjent grupy A: średnica naczyń po koło minucie od podania kapsaicyny (t_1), pow. 200×



a

b

Fig. 2a, b. Group A patient: the vessel diameter and capillary tortuosity returned back to the initial values (t_2), magnification ×200

Ryc. 2a, b. Pacjent grupu A: średnica naczyń i ich przebieg powróciły do wartości wyjściowych (t_2), pow. 200×

in the oral mucosa [2]. A lot of observations appear to indicate that the presence of capsaicin-sensitive sensory small-diameter fibres is indispensable to the development of neurogenic inflammation [6].

Goltz in 1874 proved for the first time vasodilation following sensitive fibre stimulation [21]. Two years after Striker (1876) got similar data after stimulating the spinal cord dorsal root. In 1901 Bayliss confirmed that when sensitive afferent primary fibres are electrically stimulated, a vasodilation is induced, which can only be solved by dorsal gangliotomy after 14 days from the nervous stimulation [21]. In 1967 Jancsò too proved plasma overflow after the sensitive nerve stimulation [12].

The inflammatory symptoms induced by local capsaicin stimulation are produced by the effects of the neuropeptides that are released at the peripheral sensory nerve terminals. This is proved by the fact that denervation of the area fully prevents the development of inflammatory reactions in rats [12]. Capsaicin, as a substance able to experimentally induce a NP, when administered in controlled doses lead to specific effects which could be correlated with the previously considered neuromodulator release, mainly with CGRP [19, 22, 23]. Capsaicin leads to a concentration-dependent CGPR release increase, that is at the presence of capsaicin, a higher quantity of CGPR was present at the tissue level, leading as a consequence to an increase of the blood overflow and to the main typical signs of phlogosis [6]. Another important information, mainly depending on the possible activity mechanism, is that the possible CGPR release induced by capsaicin seemed to depend on the absence of extra-cellular calcium [23].

Mainly studies proved that CGPR is often co-localised on the SP in the C-sensitive fibres, and that at the oral cavity level, the rich CGPR localisation in the trigeminal ganglions, would widely justify the role of such a substance at the oral cavity phlogosis level [1, 23].

Nerves involved in the NP pathogenesis mainly are type C, amelynic, polymodal, afferent, capsaicin-sensitive and small fibres [3]. Blood flow changes at the different organ level and the increase of the vessel permeability typical of NP mainly depends on the release of vasoactive agents at the peripheral nervous termination level, but even on other cellular components [24, 25].

However, up to now the role but mainly the pathophysiological mechanisms of the different involved mediated in the NP development were not clear yet. In fact, one of the many doubts mainly dealt with the direct activity mechanism involved, that is the activity of any released neuropeptides on the vessel plain muscle cells which would lead to their contraction and as a consequence to their diameter change, or an indirect mechanism, mediated by histamine release which then would lead to the inflammatory symptoms [26]. The recent re-definition of the SP central role mainly depended on gen-

Table 2. The characteristics of the labial microcirculation in group A and B patients

Tabela 2. Charakterystyka mikrokrążenia wargowego u pacjentów z grupy A i B

	Group A patients (Pacjenci z grupy A)		Group B patients (Pacjenci z grupy B)	
	score	subjects	score	subjects
Orientation with respect to the surface (Orientacja względem powierzchni)	A	100%	A	100%
Capillary tortuosity t_0 (Krętość naczyń dla t_0)	0 1 2	9 4 2	0 1 2	8 4 3
Capillary tortuosity t_1 (Krętość naczyń dla t_1)	0 1 2 3	0 1 7 7	0 1 2	7 5 3
Capillary tortuosity t_2 (Krętość naczyń dla t_2)	0 1 2	8 4 3	0 1 2	6 5 4
Capillary tortuosity t_3 (Krętość naczyń dla t_3)			0 1 2	7 6 2
Microhemorraghes (Mikrokrwotoki)	0	100%	0	100%
Calibre of the capillary loops – μm (Wielkość pętli naczyń – μm) (mean ± SD)	t_0 13.39 ± 0.78 t_1 21.3 ± 1.64* t_2 13.53 ± 0.95		t_0 12.96 ± 1,11 t_1 12.6 ± 1.41 t_2 13.21 ± 0.95 t_3 13 ± 1.09	

SD – standard deviation.
Differences between group A and B patients were tested by ANOVA Test.
* – significant.

SD – odchylenie standardowe.
Różnice między grupami A i B testowano za pomocą ANOVA.
* – istotne statystycznie

eral studies on rats and SP-antagonist [23, 25, 27]. It is thus possible to come to the conclusion that peptide nervous fibre are involved in the local regulation of the blood flow, in the vessel permeability and in the plain muscle tone; in real substances such as SP or C are directly involved in such mechanisms.

In presented study, capsaicin was used, a unique substance which can lead to NP at the application site and in the surrounding areas, on the oral mucosa of 30 patients.

The group A patients, results showed a powerful local stimulating effect induced by the substance, which in the majority of patients lead, after the application, to a pyrotic and painful sensation to heat. Such symptoms mainly depend on the inflammatory neuromodulator release by peripheral nervous terminations followed by a consequent vasodilation, by the increase of the capillary permeability finally leading to erythema, increase of temperature, oedema and pain. The capsaicin effect, however, is transitory and reversible. In fact, the vessel diameter measured after 45 minutes from the product application highlighted that capillaries were back to their original sizes.

The group B patients results showed a not significant modification of the diameter of the capillary loops after local anaesthetic injected. This indicates that the capillary modifications observed in the group A patients are principally due to NP. The NP is observable in the human labial mucosa.

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Received: 26.07.2004
Revised: 13.12.2004
Accepted: 13.12.2004

Praca wpłynęła do Redakcji: 26.07.2004 r.
Po recenzji: 13.12.2004 r.
Zaakceptowano do druku: 13.12.2004 r.