Effect of Capsaicin on Proliferation and Wound Healing of Dental Pulp Cells In Vitro

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Abstract

Aim of the study was to evaluate the effect of capsaicin on the proliferation and wound healing in dental pulp cells. A clonal cell line derived from dental pulp of the rat incisor was treated with capsaicin solutions of 1 nM - 10 µM, prepared from capsaicin gel. Cell proliferation and cytotoxicity were evaluated by cell counting and using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimeter assay. Areas of cell migration towards the wound scratch were measured to determine its capability on wound healing. While the 10 µM capsaicin solution expressed very high cytotoxicity, the lower concentrations (1 nM - 0.1 µM) showed a significant increase in cell viability on days 2 - 7 (P < 0.05).

Dental pulp cells treated with 0.01 µM capsaicin solution migrated towards the scratched wound more widely (P = 0.03), compared to the control. Capsaicin of lower than 1 µM is non-toxic to dental pulp cells and able to stimulate proliferation and migration of the dental pulp cells, suggesting its benefit on healing dental pulp injury.


Keywords: Capsaicin, cell proliferation, dental pulp, wound healing.

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Introduction

Capsaicin (8 - methyl - N - vanillyl - 6 - nonenamide) is an active component of chili peppers, belonging to the genus Capsicum, which are widely used in many cuisines especially in Asian countries. Besides its nutritional values, capsaicin has been claimed for its pharmacological properties, such as analgesia, anti-inflammation, antimicrobial effects and anticancer effects.¹

For decades, the use of capsaicin, as an analgesic agent in the treatment of painful disorders ranging from viral and diabetic neuropathies to rheumatoid arthritis, has been widely accepted.² Capsaicin selectively stimulates nociceptive sensory neurons and has been used to study pain mechanisms extensively. It has been shown that capsaicin modulates pain through the capsaicin receptor, which is known as transient receptor potential vanilloid subfamily member 1 (TRPV1).³ Apart from capsaicin, TRPV1, expressed in nociceptive neurons, responds to noxious heat (> 43°C) and acidic conditions (pH < 6.6).⁴ However, upon prolonged exposure to capsaicin, TRPV1 activity seems to decrease, resulting in desensitization of TRPV1 that is thought to cause the paradoxical analgesic effect of capsaicin.

Regarding anti-inflammatory effects, capsaicin has been shown to increase heme oxygenase-1 protein expression, inhibiting nitric oxide production and inducible nitric oxide synthase protein expression in macrophages.⁵ In an animal experiment, applying capsaicin cream, containing Capsicum annuum L. extract, was able to significantly decrease inflammatory swelling and reduce white blood cell count. It was explained that anti-inflammatory mechanisms of capsaicin may have come from increasing local microcirculation and decreasing porosity of blood vessels.⁶

A variety of chili pepper extracts have been proved for their antimicrobial properties on gram-positive and gram-negative pathogens.⁷ An in vitro study demonstrated antimicrobial and
anti-virulence activities of capsaicin against *Streptococcus pyogenes* (Group A streptococci), a major human pathogen, likely through inhibition of cell invasion and haemolytic activity. The antimicrobial properties of capsaicin are well considered very beneficial for medical purpose.

Capsaicin has an in vitro antiproliferative effect on prostate, colon, gastric, hepatic, lung, breast and leukemic cancer cells, while leaving normal cells unharmed. The suppression in growth of these various cancer cells occur via induction of cycle arrest, apoptosis, autophagy, and/or via the inhibition of cellular metabolic activation. Moreover, capsaicin is shown to inhibit migration of melanoma and breast cancer cells.

Despite the antiproliferative effect on cancer cells, capsaicin seems to have different effects on cell viability depending on cell types. Bone mesenchymal stem cells after exposure to capsaicin showed reduction in cell viability and proliferation via 2 pathways; capsaicin induced cell cycle arrest and increased apoptosis. Hepatic stellate cells, the major cell type involved in formation of scar tissue in response to liver damage, were decreased in number, when treated with capsaicin. On the contrary, capsaicin potentially increased airway smooth muscle cells (ASMCS) proliferation through stimulating TRPV1 activity and inhibiting apoptosis. The proliferation of gingival epithelial cells (GECs) treated with capsaicin was also increased, with a conclusion that functional TRPV1 expressed by GECs was involved in the regulation of cell proliferation.

Dental pulp is mainly composed of undifferentiated mesenchymal cells, fibroblasts, and odontoblasts, which have their healing capability. Once dental pulp is injured, treatment on the dental pulp becomes necessary, in order to eradicate the infection and heal the remaining one. Researchers have been searching for any supplement that could well promote dental pulp healing. With its pharmaceutical properties, capsaicin may be beneficial during the process of dental pulp healing. Previously, in vivo study revealed, the anti-inflammation effect of capsaicin in animal dental pulp. Nonetheless, there were few evidences on cytotoxicity and wound healing activity of capsaicin on dental pulp cells. Our study was aimed to assess in vitro the toxicity of capsaicin to dental pulp cells as well as its effect on dental pulp wound healing, if any.

### Materials and methods

#### Preparation of capsaicin solution

A local commercial product, capsaicin gel (Government Pharmaceutical Organization, Bangkok, Thailand) was used in this study. Capsaicin gel contains 0.25 g of capsaicin in 100 g of base gel. The 1.4 g of capsaicin gel was dissolved with a volume of 10 ml Dulbecco’s modified Eagle’s medium (DMEM) and filtered by 0.22 µm syringe filter to obtain 100 µM capsaicin solution. The 100 µM capsaicin solution was diluted with DMEM to make different concentrations of 1 nM, 0.01 µM, 0.1 µM, 1 µM and 10 µM capsaicin (w/v), before being used.

#### Rat dental pulp cell culture and treatment

RPC-C2A cells, a clonal cell line derived from dental pulp of the rat incisor, were used in the study. RPC-C2A cells were cultured in DMEM, supplemented with 10% fetal bovine serum, NaHCO₃ and penicillin at 1 x 10⁵ units/ml, and incubated at 37°C in humidified atmosphere of 95% air and 5% CO₂. The culture medium was changed every 2 days. In the treatment groups, RPC-C2A cells were treated with 1 nM, 0.01 µM, 0.1 µM, 1 µM and 10 µM capsaicin solutions for 24 hours, while cells in the control group were treated in the same way with DMEM containing no capsaicin. The cells were further investigated for cell proliferation, cytotoxicity. Except for wound healing assay, the treatment with capsaicin occurred after the scratched wound of cultured cells had been formed.

#### Cell proliferation

Cell proliferation was evaluated by cell counting. The seeding density was 9x10⁵ cells per well in a 24-well plate containing 500 µl of culture medium. On days 1, 2, 4 and 7, the cells from each well were trysinized with 500 µl of 0.25% trypsin for 2 minutes. After removal of trypsin, the cell suspension was made by adding 500 µl of culture medium, and pipetted thoroughly before 10µl of the cell suspension was transferred to Eppendorf micro-tube. The 10 µl of 0.4% trypsin blue solution was added and well mixed. Then, 10µl of the mixed suspension was drawn and applied to the counting chamber of hemocytometer for cell counting.

### Cytotoxicity: MTT assay

Cytotoxicity test was assessed by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma,
USA). To evaluate the number of viable cells, the seeding density was 1.6x10^5 cells per well in a 96-well plate containing 100 µl of culture medium. On days 1, 2, 4 and 7 after the treatment with capsaicin solutions, the cells were tested with MTT assay. Briefly, 50µl of MTT solution was added into each well and incubated for 2 hours after rinsing with 100µl of PBS. Then, 100µl of isopropanol was added into each well and placed on a shaker for 30 minutes. The absorbance was measured at 570 nm by microplate reader.

**Wound healing assay**

Cell migration was observed under the wound healing model on monolayer culture. A confluent monolayer of RPC-C2A cells was cultured on 24-well plates at an initial seeding density of 5x10^4 cells in 500 µl of medium per well. Cells were incubated for 24 hours. Then, a wound was created in the culture cells by gently scraping with a 1,000 µl-sized sterile pipette tip. The scratched wound areas were observed under a converted microscope and taken pictures at the beginning of time zero (T0). The width of wound areas was measured with Image Pro-Plus software (Media Cybernetics), and accepted when it was 1.2 mm ± 50µm wide.

After the wound was created, the cells were treated with capsaicin solutions (concentrations of 1 nM, 0.01 µM, 0.1 µM and 1 µM (w/v)): the 10 µM capsaicin solution was skipped due to its extensive cell toxicity, previously found in the cytotoxicity study. Cells were incubated for 8 hours, in order to allow the remaining cells to migrate towards the scratched wound area. When migration time elapsed, the whole cells on the wells were fixed with methanol solution and stained with 2% Giemsa solution (Merck, Germany). The scratched wound areas in the middle of the plate with the length of 2.74 mm were photographed and analyzed by measuring the area that the cells had migrated beyond the beginning scratch line at T0, using Image Pro-Plus software. Each experiment was performed in triplicate.

**Statistical analysis**

Statistical analyses were performed by using SPSS software (Version 18.0, Standard Software Package Inc., U.S.A.). Data of cell proliferation, cytotoxicity and migration areas in wound healing assay were presented as the mean ± SD. Kolmogorov Smirnov test was used to find out the normality of the data, and differences of the means between the control and capsaicin-treated groups were assessed with the Student’s t-test at a significant level of 0.05.

**Results**

**Cell proliferation**

No viable cells were detected in the 10 µM group throughout all periods of observation. The results of cell proliferation after treated with capsaicin solutions (1 nM - 1 µM), evaluated by cell counting, are shown as in Figure 1. On days 1 and 2, all capsaicin-treated groups except the 10 µM showed similar numbers in cell counting, compared to the control group. On days 4 and 7, cell numbers in the 1 µM group were apparently decreased compared to the control with statistical difference (P = 0.004). However, cell numbers of the 1 nM group were found significantly higher (P = 0.000) on day 7.

![Figure 1. Cell proliferation shown as numbers of RPC-C2A cells after treated with capsaicin solutions (1 nM, 0.01 µM, 0.1 µM and 1 µM) during days 1-7. The cell number in the 1 nM group is higher on day 7, while those of the 1 µM are lower on days 4 and 7, when compared to the control. ** P<0.01 vs. control.](http://www.jidmr.com)

**Cytotoxicity**

The cytotoxicity of capsaicin to the RPC-C2A cells, using MTT assay was shown in Figure 2. Along the observation periods, the 10 µM group exhibited strongly cytotoxic effect on the RPC-C2A cells, whereas the lower concentrations of capsaicin solutions were non-toxic. On day 1, MTT assay showed similar mean values of optical density absorbance for all groups. On days 2 and 4, significant increase in absorbance values of the 0.1 µM, 0.01 µM and 1 nM capsaicin groups were found (P < 0.05),
while the 1 µM group had lower value than the control without statistical significance. The result of day 7 showed the similar values for all groups, except for the 10 µM group.

Figure 2. Mean values of absorbance of the MTT assay show cytotoxicity of capsaicin after RPC-C2A cells were treated with capsaicin solutions (1 nM - 10 µM) during days 1-7. The values of the 10 µM group are very low, showing the cytotoxicity, while those of the 1 nM – 0.1 µM are higher than the control on days 2 and 4.

*P < 0.05; **P < 0.01 vs. control.

Table 1. Measurement of the migration areas after 8 hours towards the scratch zones.

<table>
<thead>
<tr>
<th>Migration area (mm²)</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Control</td>
<td>1.45 ± 0.26</td>
</tr>
<tr>
<td>Capsaicin 1 nM</td>
<td>1.08 ± 0.15</td>
</tr>
<tr>
<td>Capsaicin 0.01 µM</td>
<td>2.12 ± 0.25</td>
</tr>
<tr>
<td>Capsaicin 0.1 µM</td>
<td>1.28 ± 0.15</td>
</tr>
<tr>
<td>Capsaicin 1 µM</td>
<td>0.82 ± 0.35</td>
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</tbody>
</table>

Table 1. Measurement of the migration areas after 8 hours towards the scratch zones.

SD: standard deviation.
*Significant difference at p<0.05 when compared with the control.

Wound healing assay

Wound healing was assessed by measuring the areas in which cell migration had occurred. After treated with capsaicin solutions for 8 hours, the migration of RPC-C2A cells towards the scratched wound area could be observed obviously under a microscope in every treatment group, as shown in Figure 3. The measurement of the migration areas, shown in Table 1, revealed that the 0.01 µM group had a wider migration area (2.12 ± 0.25 mm²) with a statistical significance (P = 0.03). However, other capsaicin groups did not exhibit a significant difference, compared to the control.

Figure 3. Micrographs of the RPC-C2A cell migration towards the scratched wound at 8 hours (T8) after treated with capsaicin solutions (1 nM - 1 µM). All groups show cell migration from the beginning time (T0) towards the scratched wound and the wound areas are decreased at T8. Dotted lines indicate the beginning scratch line at T0.

Discussion

The application of capsaicin on oral health has increased recently. Due to its analgesic effect, capsaicin is used to treat neuropathic pain in trigeminal neuralgia and temporomandibular disorders. Ingestion of capsaicin is suggested to stimulate salivary secretion and enhance swallowing reflex in dysphagia patients. However, its use on dental pulp treatment remains very rare due to lack of scientific evidence on the effect of capsaicin on dental pulp cells. In our present study, we investigated cell proliferation by counting the viable cells on the plate after capsaicin treatment and its cytotoxicity using the MTT assay, in order to find out the effect of capsaicin on dental pulp cells and its appropriate concentrations to be tested on wound healing assay.
The capsaicin concentration of 10 µM, the highest in our study, caused total cell death at all time of observation, while other lower concentrations (≤1 µM) could keep normal cell viability with the increased cell numbers at specific times (Fig. 1 and 2). These suggest strong cytotoxicity of 10 µM capsaicin to dental pulp cells, when lower concentrations (1 nM, 0.01 µM and 0.1 µM) of capsaicin are likely to be non-cytotoxic and somehow promote cell viability that was observed on days 2 and 4. Our results on cell proliferation and cytotoxicity seem to agree with previous studies of ASMCs and GECs that low concentration (1 µM) of capsaicin was non-cytotoxic and significantly increased cell proliferation.22,23 Nevertheless, our RPC-C2A cells are likely a bit more sensitive to capsaicin than these cells, giving some decreased cell numbers when treated with 1 µM capsaicin on days 4 and 7 (Fig.1). This can happen due to difference in cell characteristics and cell type. Known as a capsaicin receptor, it was explained that functional TRPV1 expressed by ASMCs and GECs may contribute to the regulation of cell proliferation by capsaicin.22,23 Some have demonstrated that human dental pulp cells including odontoblasts also expressed heat/mechanosensitive TRP channels, including TRPV1,31-33 leading to the possibility that it plays the same role in regulating cell proliferation of dental pulp cells when treated with capsaicin.

Interestingly, it has been evidenced that activation of TRPV1 in sensory neurons can evoke the release of neuropeptides, including substance P and calcitonin gene-related neuropeptide (CGRP), which are major initiators of neurogenic inflammation34,35 and likely contribute to peripheral sensitization in the trigeminal sensory system and dental pain.36,37 This brings us a question of whether capsaicin can modulate inflammation of the dental pulp cells via TRPV1 activation and the release of proinflammatory mediators.

There are several reports on its anti-cancer effect both in vitro and in vivo. High concentrations of capsaicin solution (100-350 µM) showed cytotoxicity to many kinds of cancer cells, such as tongue,38 prostate,9 colon,13 gastric11 and breast cancer cells.14 Furthermore, even low concentrations of capsaicin (0.1-10 µM) were shown to prevent formation of pre-cancerous lesions in mammary organ cultures.14 The anti-cancer effect was explained to occur via induction of cell cycle arrest, apoptosis, autophagy, and/or via the inhibition of cellular metabolic activation.17,13,14,38 However, despite the anti-proliferative effect, Lui et al. demonstrated that low concentrations of capsaicin (0.1-10 µM) enhanced cell proliferation of HCT116 human colon carcinoma cells by up-regulating tumor-associated NADH oxidase.39 Data from these studies suggest that capsaicin may exert dual effects – anti-proliferative or proliferation promoting - depending on the cellular targets of capsaicin and the molecular mechanisms induced by capsaicin.

Regarding wound healing assay, we chose to examine cell migration over the wound area using two-dimensional mono-layer model as described by Shin et al. and Takahashi et al., due to its simplicity and reliability.19,23 In our study, capsaicin did not inhibit cells migrating towards the wound area in every treated group (1 nM-1 µM). Interestingly, 0.01 µM capsaicin treated group was very likely to have a stimulating effect of migration on RPC-C2A cells, while others could maintain their cell migration covering over the wound area. At first, we also concerned about the influence of cell proliferation overcoming our cell migration results. But considering the fact that none of the capsaicin-treated groups (1 nM-1 µM) showed different numbers in cell counting and absorbance value of the MTT assay at 24 hours, it is unlikely that, at the observation time of cell migration (8 hours), any enhanced proliferation of the cells could overcome the results of cells migrating towards the scratched wound.

Promotion of cell migration induced by capsaicin has been also reported in human hepatoblastoma (HepG2) cells. Low concentrations (≤0.1 µM) of capsaicin showed stimulatory cell migration of HepG2 cells, while migration was not affected with some higher concentrations of capsaicin (≥1 µM).40 A moderate activation of TRPV1 channels were explained to stimulate cell migration via modification of the microtubule dynamics of the cells, whereas disruption of the microtubule network initiated by higher concentrations of capsaicin may cause the inhibition of cell migration. Others studies expressed the inhibition of cell migration in melanoma cells and normal hepatic stellate cells induced with high concentrations (100-200 µM) of capsaicin.19,21

Cell proliferation and migration play an
important role in several physiological processes including wound healing. Our results on stimulatory effects of capsaicin on proliferation and migration of dental pulp cells have provided the possibility of clinical use of capsaicin on vital dental pulp therapy.

Conclusions

In our study, it is demonstrated that higher concentration (10 µM) of capsaicin is toxic to dental pulp cells, while lower concentrations (<1 µM) of capsaicin are non-toxic and able to stimulate proliferation and migration of the dental pulp cells. This suggests that capsaicin is likely to be a beneficial effect on dental pulp wound healing.

Acknowledgments

The authors report no conflict of interest.

Declaration of Interest

The authors report no conflict of interest.

References