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In Vivo Assessment of a Capsaicin-Containing Microemulsion for Neuropathic Pain Management

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Abstract

BACKGROUND/AIMS: Capsaicin (CAP) has been used in clinical applications for the treatment of neuropathic pain (NP). The main disadvantages of CAP are its short half-life, low water solubility and low bioavailability. This study intended to produce a therapeutically effective microemulsion (ME) formulation comprising CAP to decrease NP.

MATERIALS AND METHODS: ME was prepared using oleic acid, Tween 80, propylene glycol, ethanol, and water. Neuropathy was induced by partial sciatic nerve ligation (PSL) in mice. Two weeks after, PSL NP was tested using the cold plate (CP) and von Frey (VF) tests. The mice in the treatment group were administered 10 mg/kg CAP by oral gavage. The effects on NP of conventional CAP and ME CAP were compared.

RESULTS: The prepared ME formulation of (CAP) was a homogeneous, transparent, thermodynamically stable dispersion of water and oil. The classic CAP was not effective on NP, while ME CAP was effective in the CP. The ME CAP was more effective on mice, than classic CAP using the VF test.

CONCLUSION: The developed novel ME formulation at lower doses could reduce side effects and improve the bioavailability of the oral administration of CAP in the treatment of NP, and thus, would achieve good patient compliance.

Keywords: Capsaicin, mice, microemulsion formulation, neuropathic pain

INTRODUCTION

Neuropathic pain (NP) is an inappropriate response caused by a primary lesion or dysfunction in the nervous system. It is a chronic type of pain that is difficult to treat and does not respond to known analgesics. Since NP may not respond in part or at all to common analgesic therapies, adjuvant analgesics such as antiepileptics, antiarrhythmics, and antidepressants are widely used in the medical treatment of NP.[1] As a second line therapy, capsaicin (CAP) is recommended for NP.[2] CAP is an

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active ingredient found in chili peppers and similar plants of the capsicum plant family, and has long been used as an analgesic agent.[3] There are preparations of CAP obtained from hot red pepper that can be applied to the skin as creams and patches. On the other hand, CAP has some disadvantages. The main disadvantage of CAP is its short half-life and low bioavailability. Another significant drawback of using it as a medication is the burning sensation, which negatively affects patient compliance.[4] For this reason, various strategies such as hydrogel formation, encapsulation in liposomes, iontophoresis, and sustained-release formulations were developed to eliminate these disadvantages and ultimately increase its bioavailability. In addition, the development of drug delivery systems has intensified in recent years to enhance the bioavailability of CAP and increase its therapeutic index.[5] Drug delivery systems have generally been developed for transdermal and oral administration of CAP.[6] In the past years, microemulsion (ME) systems have been widely studied for their superior advantages such as ease of preparation, homogeneity, improved solubility, and permeability. MEs are transparent, thermodynamically stable dispersions of oil, water, and surfactant (S), often significantly combined with a cosurfactants (CoS). The oil-in-water (o/w) MEs seem to be promising because they can dissolve components with poor water solubility in the oil phase, enhancing their solubility.[7] Therefore, in this study, we aimed to develop an orally effective delivery system, o/w MEs, in order to increase the solubility and in *vivo* bioavailability, and to decrease adverse effects of CAP.

MATERIALS AND METHODS

Chemicals

CAP (Sigma Aldrich, Cas no:404-86-4) as an active agent, oleic acid (Sigma Aldrich, Cas no:112-80-1) as an oil phase, Tween® 80 (Sigma Aldrich, Cas no:9005-65-6) as a S, propylene glycol (Sigma Aldrich, Cas no:57-55-6) and ethanol (Sigma Aldrich, Cas no:64-17-5) as co-S and water as an aqueous phase.

Ternary Phase Diagram Construction

The preparation of a self-emulsifying system used pseudo-ternary phase diagrams of MEs. The regions such as the ME and nanoemulsion, which vary according to the amount and ratio of oil, water, and S, were determined on the pseudo ternary phase diagrams.[8] Different concentrations of S and CoS were studied with oleic acid, Tween® 80, propylene glycol, and ethanol. Tween® 80 was used as a S while propylene glycol and ethanol were selected as the CoS in an S/CoS weight ratio of 2:1, 3:2, and 3:1.[9]

Characterization of Formulation

Globule size, zeta potential, polydispersity index (PDI), and pH were measured to determine the physicochemical properties of ME. Zeta potential and globule sizes of MEs were measured using Zetasizer nano ZS (Malvern, United Kingdom) at 25 °C, and the PDI was reported. results Samples were placed in clear, one-use zeta cells, and results were obtained.[8] After preparing the MEs, the pH value of those emulsions was measured.

Pharmacological Evaluation

25-30 g Swiss albino male mice, obtained from Çukurova University Health Sciences Experimental Application and Research Center, were used. Approval was obtained from the Çukurova University Experimental Animals Ethics Committee (approval number: 4, date: 08.07.2019). Partial sciatic nerve ligation was performed to produce neuropathy, as described previously in rats by Seltzer et al.[10] In sham-operated mice, the nerve was exposed, but not ligated. NP has tested two weeks after sciatic nerve ligation using the cold plate [(CP), cold allodynia] and von Frey [(VF), mechanical allodynia] tests. CP analgesiometer (Ugo Basile, Hot/CP Analgesia, Italy, Cat. No. 35150) and VF filaments (Ugo Basile, VF Hairs, Semmes-Weinstein set of monofilaments, Code: 37450-275) were used for CP and VF tests, respectively. In the CP test, mice were placed on a metal plate at 5 degrees and their reaction times [CP latency (CPL)] were measured.[11] In the VF test, the filaments are applied on the plantar surfaces of mice's feet with a series of increasing forces until the animal gives a reaction [VF Threshold (VFTH)].[12] A dose of 10 mg/kg (ME CAP), and a dose of 10 mg/kg classic CAP, were administered via oral gavage, 2 h before the tests. The placebo (control) group was given a placebo without active pharmaceutical ingredients.

Statistical Analysis

Variables were summarized as mean and standard deviation. For comparison of groups, One-Way ANOVA was used. Tukey's Games-Howell tests were used for multiple comparisons of groups regarding the homogeneity of variances. IBM SPSS Statistics Version 20.0 statistical software package was used to perform all analyses. The statistical level of significance for all tests was considered to be 0.05 (IBM Corp. Released 2011).

RESULTS

The ternary phase diagram describes the ideal experimental conditions, for putting the components together to form a clear preparation. The S and CoS were weighed at different ratios (2:1, 3:2 and 3:1) in each tube. The pseudo-ternary phase diagram has been created by means of a computer program. The percentage of ME area in most of phase diagrams was largest at an S/CoS weight ratio of 2:1, compared to others (Table 1). The prepared blank and CAP -loaded MEs were clear, transparent, liquid, single-phase, free of drug precipitation, and homogeneous in appearance. Table 1 shows the physicochemical parameters of MEs in the presence and absence of CAP.

CPL and VFTH of the groups were measured two weeks after the neuropathy and sham operation. ME CAP and classic CAP were administered by oral gavage 2 hours before the tests. According to results, CPL and VFTH of the sham group were not different from the control, whereas a significant decrease was observed in the CPL and VFTH in the NP group (Figures 1, 2 respectively). In experiments examining the effects of ME CAP and classical CAP on NP, it was observed that CPL of the ME CAP group was significantly increased; however, CPL of the classical CAP group was not increased compared to

Table 1. Optimum formulation ingredients and characterization of microemulsion formulations

Code/formulation	Oil (%)	Water (%)	S (%)	CoS (%)	CAP (%)	pH	Zeta potential (mV)	Globule size (nm)	PDI
Splacebo	7.6	34.5	38.6	19.3	-	5.42±0.01	0.56±0.18	124.6±2.8	0.2±0.02
Scapsaicin	7.6	34.2	38.6	19.3	3	5.31±0.02	0.60±0.36	156.6±3.4	0.3±0.04

CoS: Cosurfactant, CAP: Capsaicin, S: Surfactant, PDI: Polydispersity index.

the NP group (Figure 3). According to these results, the ME CAP reduced NP in CP test, but classic CAP did not show any effect on NP. On the other hand, both classic CAP and ME CAP decreased NP in the VF test. Furthermore, a significant increase in VFTH was observed in the ME CAP group compared with the classic CAP group in the VF test (Figure 4). It can be suggested that ME CAP is more effective on NP than classic CAP in the VF test.

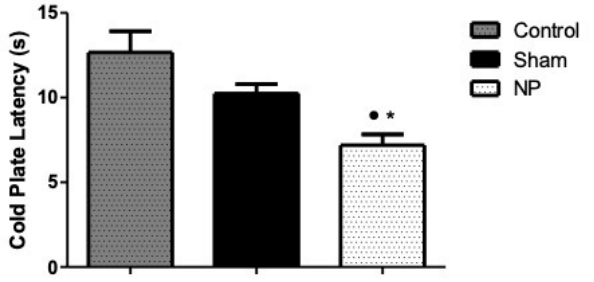

Figure 1. CPL latencies in Control, Sham and NP groups. One-way ANOVA, according to Tukey's multiple comparison test: *: Significantly different from the control group. ($p<0.05$). •: Significantly different from Sham group ($p<0.05$).

CPL: Cold plate latency, NP: Neuropathic pain.

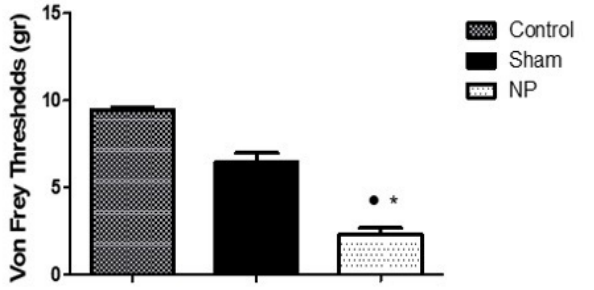

Figure 2. VFTH in control, sham and NP groups. One-Way ANOVA, according to Tukey's multiple comparison test; *: Significantly different from the control group. ($p<0.05$). •: Significantly different from Sham group ($p<0.05$).

VFTH: von Frey Thresholds, NP: Neuropathic pain.

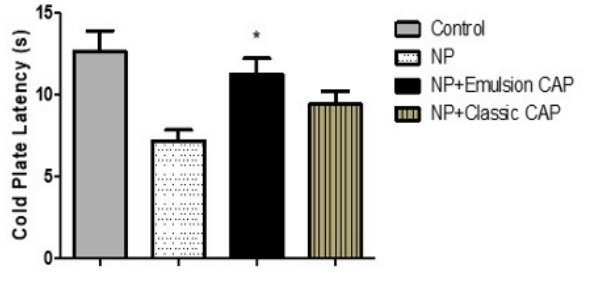

Figure 3. The effects of ME and classic CAP Formulation in the CP test. One-Way ANOVA, according to Tukey's multiple comparison test; *: Significantly different from the NP group ($p<0.05$).

ME: Microemulsion, CAP: Capsaicin, CP: Cold plate, NP: Neuropathic pain.

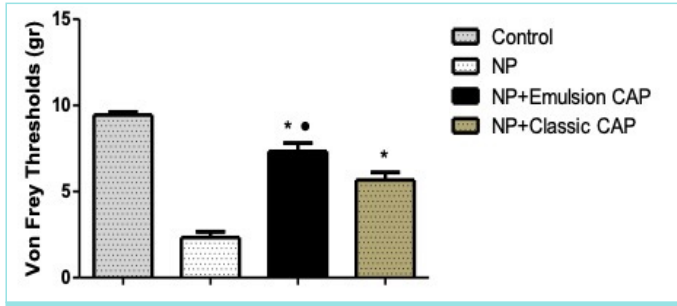

Figure 4. The effects of ME and Classic CAP Formulation in the VF test. One-Way ANOVA, according to Tukey's multiple comparison test: *: Significantly different from the NP group ($p<0.05$), •: Significantly different from NP + classic CAP group ($p<0.05$).

ME: Microemulsion, CAP: Capsaicin, VF: von Frey, NP: Neuropathic pain.

DISCUSSION

Nanotechnological drug delivery systems like MEs have been widely investigated for a variety of pharmaceutical applications. MEs, with their excellent solubilization, permeability, and bioavailability-enhancing properties, have already been applied in clinics to improve oral drug delivery.[13] Globule sizes of CAP loaded formulations were slightly larger than the blank formulations, but this difference was not statistically significant ($p>0.05$). Based on previous studies, the pH value of these formulations is suitable for oral administration, and there are no significant differences between them (Table 1). The Zeta potential value is crucial for studying emulsion stability.[14] Due to compounds like non-ionic S, the zeta potential of MEs is nearly neutral.

In a study conducted by Sethuram and Thomas[15], ME-loaded nanofibrous membranes with a homogeneous distribution were fabricated. The developed formulations demonstrated controlled and sustained release in the wound environment and were suggested to be suitable for use in wound infection treatment. In another study, baicalin-loaded MEs were found to improve transdermal delivery by enhancing solubility, skin permeation, and retention. Additionally, the formulation demonstrated good efficacy and a high safety profile in anti-inflammatory and analgesic experiments.[16]

In our study, the pSLN model, defined by Seltzer et al.[10] was used to create experimental NP. Pain behaviors observed in animals in the pSLN model are similar to many symptoms (allodynia; hyperalgesia) in humans with NP syndrome.[17] It was shown that cold allodynia and mechanical allodynia, which are the most important symptoms of NP, occur as a result of partial ligation of the sciatic nerve.[18] The use of the sciatic nerve to create experimental peripheral NP is common because it is easy to access and suitable for performing paw tests.

As a result of the CP test, there was no significant difference between the CPL of control and sham groups; however, the CPLs of the animals ligated to the sciatic nerve (NP group) were significantly lower than those of the control and sham groups (Figure 1). This result shows that cold allodynia occurs with our method and is consistent with the literature and the results of our previous studies.[19-20] In addition, ME CAP significantly prolonged CPL compared to the NP group, but no difference was observed between classical CAP and the NP group in the CP test. According to this result, ME CAP is effective for NP pain, but

classic CAP is not, and we may say that ME CAP is superior to classic CAP in the CP test (Figure 3).

In the VF test, the VFTH of the animals in the control and sham groups was similar, and there was no significant difference between them. However, the VFTH of sciatic nerve ligated mice (NP group) was significantly reduced when compared to the control and sham groups (Figure 2). This result shows that mechanical allodynia occurs with our method, and the results are consistent with the literature.[22,23] Differently from CP test both ME CAP and classic CAP groups were effective on NP in VF test. Furthermore, a significant increase in VFTH was observed in the ME CAP group compared with the classic CAP group (Figure 4). Thus, we can say that the novel ME CAP formulation is more effective than classic CAP formulation in VF test.

We found that the o/w ME CAP formulation seems to be promising because it was more effective against cold and mechanical allodynia. Probably this formulation improves the solubility, absorption, and thus bioavailability of CAP. It is reported that oral CAP ingestion may cause several side effects because it needs to be used in high doses to be effective.[24] On the other hand, topical CAP causes a burning sensation.[4] So, our study provided the opportunity to develop a preparation suitable for oral use. These results show that the new oral formulation prepared in the study can be used at lower doses, thus having fewer side effects and providing better patient compliance.

Study Limitations

In this study, although we planned to investigate the effectiveness of CAP in NP by preparing new oral and topical formulations, only the oral formulation was prepared and its effectiveness was examined. The main purpose of the study was to prepare a more effective oral formulation. If there was no response to the oral formulation, we considered the topical formulation, as plan B. Since the expected results were obtained from the oral formulation we prepared, the topical formulation was not studied, in order not to use more animals in terms of animal ethics.

CONCLUSION

While classical CAP was not effective against cold allodynia due to NP, the new oral CAP formulation was found to be effective, which was the purpose of this study. Furthermore, the new formulation was found to be more effective against mechanical allodynia due to NP than the oral form currently used. Based on these results, the effects of the new formulation we have prepared warrant further detailed examination with additional studies. In conclusion, the developed ME formulation improves the solubility of CAP, enhances its oral bioavailability, and is a promising basis for further development as a formulation for oral administration.

MAIN POINTS

- By using a titration method with a small particle size and polydispersity index range, a capsaicin (CAP)-loaded microemulsion (ME) can be successfully prepared.
- Classical CAP is ineffective against cold allodynia, but is effective against mechanical allodynia.
- CAP prepared in ME form is effective against both cold allodynia and mechanical allodynia.
- The ME formulation of CAP is more effective than classical CAP in the treatment of neuropathic pain.
- The oral efficacy of drugs can be increased by administering them in ME form.

ETHICS

Ethics Committee Approval: Approval was obtained from the Çukurova University Experimental Animals Ethics Committee (approval number: 4, date: 08.07.2019).

Informed Consent: Not available.

Footnotes

Authorship Contributions

Surgical and Medical Practices: R.S., A.A., S.E., Concept: R.S., U.M.G.B., S.D.K., F.A., Design: R.S., U.M.G.B., S.D.K., F.A., Data Collection and/or Processing: R.S., U.M.G.B., A.A., Analysis and/or Interpretation: U.M.G.B., S.D.K., F.A., Literature Search: R.S., U.M.G.B., S.D.K., S.E., Writing: R.S., U.M.G.B., S.D.K., F.A.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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