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Antiallodynic and Antihyperalgesic Activities of Fentanyl-Loaded Dermal Clay Dressings in Rat Model of Second-Degree Burn Injury

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ABSTRACT

Second-degree burn injury is the most common type of burn injury, which usually takes 2-3 weeks for complete healing. However, such patients suffer with intense pain associated with development of hyperalgesia and allodynia. Here, we prepare a silver clay patch using montmorillonite clay, betaine, and silver nitrate. Later, the silver clay patches were loaded with fentanyl. Furthermore, the patches were fabricated into burn wound dressings. The dressings were first subjected to *ex vivo* skin penetration studies and were later evaluated for thermal hyperalgesia and mechanical allodynia using second-degree burn injury rodent model. Our results show that application of fentanyl-loaded dermal clay (FLDC) dressings for 3 h showed significant increase of paw withdrawal latency (p <0.001) against hyperalgesia starting from 30 min after removal of patch to up to 6 h. Similarly, the FLDC dressings also potentiated the paw withdrawal threshold for up to 4 h after application (p <0.001). From these studies, we can conclude that FLDC dressings are ideal topical formulations for better management of pain in second-degree burns.

Introduction

In spite of significant advances in pain research, the pain management in burn victims is still evolving.[1] This is mainly because of both the complex nature of pain and insufficient understanding of the mechanisms and potential approaches to mitigate the pain.[2,3] Injury to peripheral nerves often results in hyperalgesia (pain evoked by a noxious thermal stimulus) and allodynia (pain evoked by an innocuous mechanical stimulus).[4,5] Allodynia and hyperalgesia are seen at the site of burn and also in areas of close proximity to the injury.[6-8] These debilitating pain components severely affect the physical activities of the patients and may further lead to emotional distress.[9] Opioids are the first line of therapy in the pain management today despite their systemic adverse effects.[10] Fentanyl is a pure opioid receptor agonist that is widely used in chronic cancer pain and postoperative pain management. Fentanyl and its analogues in the form of extended-release formulations such as infusions and transdermal patches provide prolonged analgesic activity by maintaining sustained plasma levels.[11,12] However, on systemic administration, it has serious adverse effects. In a preclinical study, fentanyl has caused severe neurotoxicity including brain lesions and convulsions over a range of doses.[13] In addition, activation of central opioid systems leads to addiction, nausea, and respiratory depression. Because opioid receptors are widely expressed in the peripheral nervous system, locally applied fentanyl has potential in treating acute pain conditions.[14] Furthermore, it has been found that opioids themselves induce hyperalgesia referred to as opioid-induced hyperalgesia. Most studies have demonstrated that the mechanisms involved in such opioid-induced hyperalgesia predominantly arise on account of systemic administration of opioids.[15,16]

In addition to pain, burn patients are prone to infections. Hence, they have to be covered using wound dressings that offer protection against microbial infection as well. Generally, in infectious period of burn wound, an antibiotic-containing wound dressing would be preferred.[17] Clay has been used since time immemorial to heal skin disorders and wounds by maintaining optimal hydration.[18] Silver is known to possess antimicrobial activity.[19] Our earlier studies have clearly shown that silver-loaded clay patches would serve as a great wound dressing that facilitates wound

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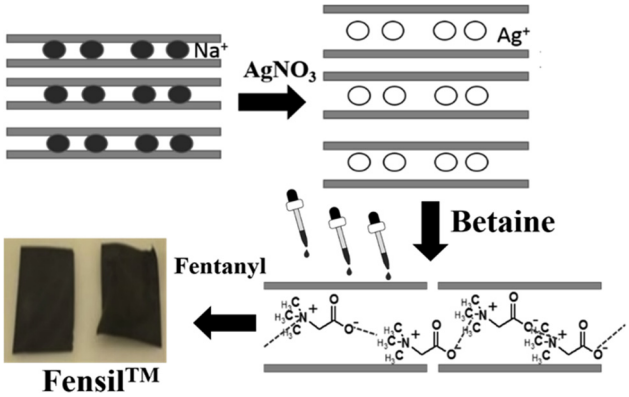

Figure 1. Schematic diagram of preparation steps involved in FLDC patch (ion exchange of silver ions, intercalation of betaine and loading of fentanyl).

healing as well as minimizes the susceptibility to infections.[20-22] In addition, clay can soothen the wound surface and would benefit the burn wounds. Thus, in this study, we first prepared the silver clay patches and loaded them with fentanyl. These fentanyl-loaded dermal clay (FLDC) patches were fabricated into wound dressings. Later, they were subjected to *ex vivo* skin penetration studies to assess the extent of drug in dermal layers. The FLDC patches were then evaluated for the hyperalgesic and allodynic activity using second-degree burn—induced rodent model.

Materials

Chemicals

Bentonite U.S. Pharmacopeia clay was procured from Charles B. Chrystal Co. (Larchmont, NY). Betaine hydrochloride and silver nitrate ($AgNO_3$ 99.9%) were purchased from Strem Chemicals Inc. (Newburyport, MA). Fentanyl was received as a gift sample from Noramco Inc. (Athens, GA).

Animals

Male Sprague-Dawley rats (Charles River, Hollister, CA), weighing 250-275 g, were housed in groups of 3 in the animal care facility at the University of Mississippi, Oxford, Mississippi, under a 12-h light/dark cycle, and were allowed access to standard rat chow and water *ad libitum*. All animals were tested during the 12-h light cycle (7 AM-7 PM) on successive days. Experiments followed American Association of Laboratory Animal Care guidelines, were approved by the Institutional Animal Care and Use Committee (IACUC) of University of Mississippi (approval no. 16-026 dated 05/24/2016), and adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain.[23] The animals were randomly divided into 4 groups of 4 animals each. Group I—plain silver clay patch vehicle control group (patch without fentanyl) was applied with silver clay patch, group II—FLDC dressing test group (FLDC patch loaded with fentanyl [80 μg/cm^2], group III—fentanyl parenteral positive control group (fentanyl solution [3 μg in 0.1 mL] administered intradermally at the site of injury), and group IV—fentanyl parenteral negative control group (fentanyl solution [3 μg in 0.1 mL] administered intradermally away from the site of injury). The FLDC dressing for group I was secured on to the burn injured paw with adhesive tape for 3 h under 4% isoflurane anesthesia.

Methods

Preparation of Silver Clay Patch

$AgNO_3$ was dissolved in deionized water in a UV-sterilized plastic bottle. Montmorillonite clay was added to the $AgNO_3$ solution and mixed in a rotary ball mill in the dark for 24 h. The weight ratio between $AgNO_3$ and clay was kept about 1:10. This provided silver loading level onto clay of about 0.5 mg/cm^2, which is a typical concentration found in most of the commercial silver-containing wound dressings[24] (weight of 1-cm^2 silver clay patch was about 0.022 g. The weight % of silver in silver clay patch is about 2.24 wt% based on energy-dispersive X-ray spectroscopy elemental analysis. Therefore, the weight of silver per cm^2 was calculated to be about 0.5 g/cm^2). The ball-milled mixture was centrifuged and washed with deionized water to remove excess silver. Anhydrous betaine hydrochloride was added to the Ag-clay solution in the weight ratio between betaine and clay of about 1:6 and was mixed again in a ball mill for 24 h. After thorough mixing of the ingredients, the solution was poured on a clean 8″ × 12″ silicone mat and cast as a thin film. Once dry, the Ag-betaine-clay film was removed from the silicone

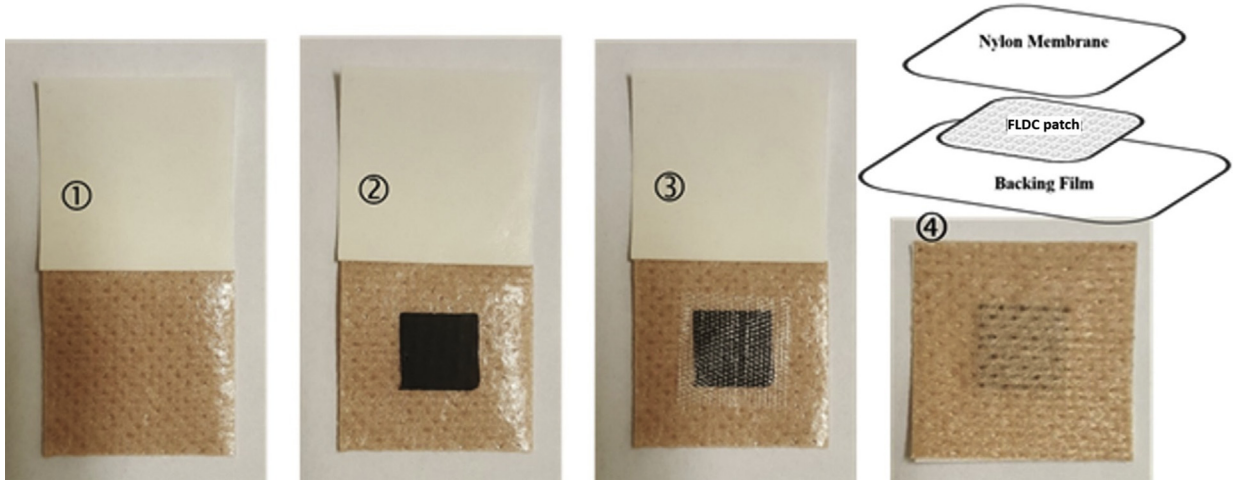

Figure 2. Fabrication steps of the prototype FLDC dressing: (1) backing membrane, (2) FLDC patch placed over the backing membrane, (3) Delrin release liner placed over the FLDC patch, and (4) prototype of FLDC dressing.