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Original Article

Influence of nanoparticle size on the skin penetration, skin retention and anti-inflammatory activity of non-steroidal anti-inflammatory drugs

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Abstract

Background: This study aims to evaluate the influence of nanoparticle size on the in vitro percutaneous penetration and retention and in vivo anti-inflammatory efficacy of percutaneously delivered non-steroidal anti-inflammatory drugs.
Methods: Indomethacin, ketoprofen and piroxicam were incorporated into nanoparticles. The nanoparticles, or the bulk-drug equivalents, were suspended in a hydrophilic ointment and compared for their ability to facilitate percutaneous drug penetration and retention in vitro. The formulations were applied cutaneously in a carrageenan-induced footpad inflammation model (acute inflammation) and an adjuvant-induced arthritis model (chronic inflammation) in rats and were assessed for their anti-inflammatory efficacy and potency.
Results: The nanoparticle formulations demonstrated a substantially smaller particle size compared with the bulk-drug formulations. The nanoparticles notably increased drug penetration and retention in vitro. In both the acute and chronic inflammation models, the nanoparticle formulations demonstrated significantly higher anti-inflammatory activity than that of their corresponding bulk-drug formulation at an equivalent dose, and produced better overall healing.
Conclusion: The nanoparticle formulations are highly effective as percutaneous drug carriers, and demonstrate that decreasing particle size leads to increased efficacy and potency. The exploitation of such nanotechnology could drive the development of more effective percutaneous therapeutics.

Keywords: Drug delivery; Nanoparticles; Nanotechnology; Non-steroidal anti-inflammatory drugs (NSAIDs); Percutaneous

1. Introduction

Nanotechnology has been described as a field aimed at creating materials with new functions and beneficial characteristics through the control of material structure/sequence at the nanometer level.[1,2] Following advances in nanotechnology, it is now becoming possible to create drug delivery systems (DDSs) using nanoparticles or microparticles as drug carriers. Percutaneous drug delivery is often used as a route of administration for low-molecular-weight drugs. For example, a percutaneous route has been adopted for the administration of non-steroidal anti-inflammatory drugs (NSAIDs) to avoid the limitations of oral administration, such as gastrointestinal symptoms and renal impairment.[3] The skin is the largest organ in the human body. If certain chemicals are applied to the skin, the active ingredient can penetrate through the skin barrier and become distributed throughout the whole body via the intradermal capillaries. However, the skin also possesses a barrier mechanism to prevent the invasion of various materials into the body.[4,5] For this reason, the penetration of materials across the skin is generally quite low, compared with penetration

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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across other tissues. The skin is composed of the epidermis (including the horny layer), the dermis, and subcutaneous tissue. Accessory structures in the skin, such as sweat glands, are also present, and span the epidermis and the dermis. Of these skin components, the horny layer has the highest barrier function. Therefore, the effective passage of drugs through the horny layer is an issue of particular focus in percutaneous drug delivery.[6–10]

NSAIDs are widely used in the clinic because of their excellent anti-inflammatory and analgesic action.[11] However, oral-dose NSAIDs are associated with adverse systemic reactions, such as gastrointestinal symptoms and renal impairment. For this reason, percutaneously absorbable forms of NSAIDs are often used when dealing with local inflammatory diseases, making locally and percutaneously absorbable drug formulations important research targets. To improve skin penetration, various penetration-promoting agents[12,13] and particle sizes[14] have been studied, and different formulations such as liposomes,[15] gels,[16–19] ointments and creams[20,21] have been investigated and utilized. The application of nanoparticles to the skin reportedly elevates drug penetration across the skin barrier. Nanoparticles are therefore expected to provide a means of improving drug penetration through the skin, and local DDSs using nanoparticles have the potential for extensive clinical use. However, differences in particle size can affect nanoparticle skin uptake, retention time during circulation, and the potential for adherence and degradation. Keeping these points in mind, we explored the potential of nanoparticles and microparticles as percutaneous drug carriers by preparing fine particles containing NSAIDs using a planetary centrifugal mixer and evaluating their skin penetration and safety.[22] In addition, the anti-inflammatory activity of the NSAID particles was evaluated in animal models of acute and chronic inflammation.

2. Methods

2.1. Materials and reagents

Indomethacin (IMC), ketoprofen (KET), piroxicam (PXC) and diclofenac sodium were purchased from Nacalai Tesque, Inc (Kyoto, Japan). Other reagents used were commercial grade.

2.2. Preparation of fine NSAIDs particles

NSAIDs were incorporated into fine particles by processing the drug, zirconia beads (2.5 g), and an aqueous polymer (hydroxypropyl cellulose SSL [HPC]) solution in a planetary centrifugal mixer (NP-100; THINKY Corporation; fitted with a −20 °C freezer) at a rotation rate of 1700 rpm, a mixing time of 10 min, and a medium (zirconia ball) quantity (2.5 g). The particle size of the drug in the suspension was measured using a laser diffraction particle size distribution meter. The active ingredients (ai) used were IMC (IMCai), KET (KETai), and PXC (PXCai), and the fine-particle formulations of these drugs are expressed as IMCnano, KETnano, and PXCnano, respectively.

2.3. Skin penetration test of fine-particle NSAIDs and measurement of their residual levels in the skin

2.3.1. Preparation of test drug ointments

NSAIDai and NSAIDnano were individually suspended in anhydrous ethanol and combined with a hydrophilic ointment. The drug concentration was 1 w/w%.

2.3.2. In vitro skin penetration of NSAID ointment

2.3.2.1. Skin penetration test[23]. The excised dorsal skin of hairless mice (Laboskin®, Hos: HR-1 male, 7 weeks; Hoshino Laboratory Animals, Inc., Saitama, Japan) was used. Franz-type diffusion cells (effective diffusional area: 1.77 cm^2, receptor volume: 12 mL) were also used. Each test drug (0.2 g) was added to the epidermal side of the Laboskin®. The receptor phase was agitated, and the temperature was maintained at 37 °C. Following the addition of the test drugs, the receptor solution (0.5 mL) was collected over time. The concentration in each sample was measured using HPLC. The skin permeation parameters were calculated using the method reported by Iwaki et al.[24]

2.3.2.2. Measurement of residual drug concentration in skin. Skin mounted on a Franz-type diffusion cell (1 cm^2) was harvested over time in a manner similar to that described in section 2.3.2.1. The harvested skin was cut into small pieces and combined with methanol for homogenization, followed by centrifugation for 30 min (15,000 × g, 4 °C). The supernatant was used as the test sample. The drug concentration in the test sample was measured using HPLC.

2.3.3. Test drug quantification method

The HPLC system used was an LC-10 System, Shimadzu Corporation (Kyoto, Japan). The concentration of NSAIDs was quantified in accordance with the Japanese Pharmacopoeia 16th edition.

2.4. Anti-inflammatory activity

2.4.1. Experimental animals

Male Wistar rats (5 weeks old) were purchased from Japan SLC, Inc. (Shizuoka, Japan). The rats were housed at 23 ± 2 °C and 50 ± 3% humidity. The animals were divided into an untreated group, a drug-free vehicle treatment group, and a treatment group for each test drug (NSAIDai group and NSAIDnano group). The experiment was performed with the approval of the Animal Experiment Review Board of Kochi University and in accordance with the Guidelines for Animal Experiments of Kochi Medical School.

2.4.2. Test drugs

Using a method similar to that described in section 2.3.1, NSAID ointments were prepared at concentrations of 0.5, 1, and 3% and a dose-response test was then conducted using these ointments. To compare efficacies, ointments with the same concentrations as those of commercially available

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