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Noninvasive delivery of siRNA into the epidermis by iontophoresis using an atopic dermatitis-like model rat

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Abstract

Topical application of siRNA to the skin should be an effective treatment for serious skin disorders, such as atopic dermatitis. However, it is difficult to introduce hydrophilic macromolecules, including siRNA, into the skin by conventional methods. For efficient delivery of siRNA, we examined an iontophoretic technique, since it is suitable for the delivery of charged molecules. Naked siRNA effectively accumulated in the epidermis (and not in the dermis) after iontophoretic delivery. In contrast, siRNA did not penetrate tape-stripped skin by passive diffusion. In a rat model of atopic dermatitis, skin was sensitized with ovalbumin to stimulate IL-10 mRNA expression as observed in skin lesions. Iontophoretic delivery of anti-IL-10 siRNA significantly reduced (73%) the level of IL-10 mRNA. In conclusion, we successfully delivered naked siRNA into the epidermis and concomitantly suppressed the expression of an endogenous immuno-regulatory cytokine.

Keywords: 
SiRNA
Transdermal delivery
Iontophoresis
Interleukin-10

1. Introduction

Small interfering RNA (siRNA) is a double-stranded molecule which can be designed to hybridize with a specific mRNA sequence. siRNA inhibits the translation of numerous genes both in vitro and in vivo (Fougerolles et al., 2007). Therefore, topical introduction of siRNA targeted against genes involved in various cutaneous disorders represents a novel therapeutic approach to the treatment of inherited skin diseases, viral infections, skin cancer and atopic dermatitis (AD). However, it is difficult to deliver siRNA into the skin by conventional methods based on passive diffusion (simple swabbing or application of a patch) because siRNA is a hydrophilic macromolecule. Recently, it was reported that intra-dermal injection or electroporation could be used to deliver siRNA directly into the skin (Wang et al., 2007a,b; Inoue et al., 2007). However, these methods are invasive or require special devices. As a noninvasive and simple alternative, iontophoresis is known to accelerate transdermal permeation of charged molecules by applying a slight current to the skin (Kalia et al., 2004). Successful delivery of hydrophilic macromolecules, such as insulin, by iontophoresis has been reported (Kanikkannan et al., 1999). Hence, we hypothesized that iontophoresis might be applicable for noninvasive delivery of siRNA into the skin. Here, we report successful iontophoretic delivery of naked siRNA into the epidermis of the rat. In addition, we investigated the effect of siRNA on endogenous levels of interleukin-10 (IL-10) mRNA, which is elevated in AD skin lesions (Ohmen et al., 1995).

2. Materials and methods

Hair was shaved off the dorsal skin of six-week-old male Brown Norway (BN) rats (Charles River, Japan), and the skin was subjected to tape stripping to remove stratum corneum and 100 µL of 0.15% (w/v) ovalbumin (OVA) solution was intradermally injected every 24 h for one week in order to induce AD-like skin lesions (Sengoku et al., 1999; Mizushima et al., 1999; Wang et al., 2007a,b). Absorbent cotton (3 cm²) containing 10 µg of Cy3-labeled siRNA (Ambion) dissolved in 200 µL of sterile water was placed on the OVA-treated skin, and a second cotton patch moistened with 200 µL of saline was placed on the skin 1 cm away. The cotton patches were connected to the cathode and anode of a power supply (TTI ellebeau Inc., model-TCCR-3005, Tokyo, Japan) with Ag–AgCl electrodes and covered with tape. Iontophoresis was conducted at constant current density, 0.3 mA/cm² (0.9 mA) for 1 h. The skin under the cathode was excised immediately after iontophoresis, and a cross section of the skin (15 µm thickness) was prepared by cryostat (LEICA, CM3000, © 2009 Elsevier B.V. All rights reserved.)
The group treated with OVA and siRNA iontophoresis was treated with tape striping, OVA treatment and siRNA iontophoresis. Rats were divided into four groups. The non-treated group never received tape striping, OVA treatment or siRNA iontophoresis. In order to increase IL-10 transcript levels in the skin, the dorsal skin of the six-week-old BN rats was treated as follows. Sixteen BN rats were divided into four groups. The non-treated group never received tape striping, OVA treatment and siRNA iontophoresis. The other three groups were subjected to tape stripping on day 0. The OVA-treated group received OVA every 24 h for one week. The group treated with OVA and siRNA iontophoresis was treated with OVA and siRNA against rat IL-10 mRNA or luciferase mRNA. All skin samples of BN rats were resected at the same time (immediately after iontophoresis for confocal study or 12 h later for total RNA isolation).

In order to increase IL-10 transcript levels in the skin, the dorsal skin of the six-week-old BN rats was treated as follows. Sixteen BN rats were divided into four groups. The non-treated group never received tape striping, OVA treatment and siRNA iontophoresis. The other three groups were subjected to tape stripping on day 0. The OVA-treated group received OVA every 24 h for one week. The group treated with OVA and siRNA iontophoresis was treated with OVA and siRNA against rat IL-10 mRNA (Qiagen, antisense; 5′-AAAUGCAUGACACGCUCCGTdTdT-3′) or siRNA against luciferase mRNA (Greiner, antisense; 5′-GGGUUGGCACCAGCAGCGCTT-3′). The siRNAs were topically applied to the OVA-treated skin site by iontophoresis as described above 24 h after the final OVA treatment (day 7). The whole skin biopsies (3 cm²) of all BN rats were isolated at the same time (12 h after iontophoresis), and total RNA was extracted by TRIzol (Invitrogen) according to the manufacturer’s procedure. The detailed time schedule for OVA treatment and siRNA iontophoresis is shown in Fig. 1. IL-10 transcript levels in the skin were determined by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis. The CDNA was synthesized by PrimeScript reverse transcriptase from 5 μg of total RNA. Amplification of IL-10 was performed with forward primer (5′-AGCTGCGACGCTGTCATCGA-3′) and reverse primer (5′-CAAGGAGTTGCTCCCGTTAG-3′) using Ex Taq Hot Start version (TaKaRa). 18S RNA was used as an internal standard and amplified with Quantum RNA Classic II 18S standards (Ambion). The PCR products were subjected to gel electrophoresis (2% agarose) and visualized by SYBR Green I (Sigma) staining. Band intensities were measured with a CS Analyzer 2.0 (Atto). Relative IL-10 transcript levels were represented as band intensities of IL-10 divided by that of 18S rRNA.

3. Results and discussion

First, we examined iontophoretic delivery of siRNA into tape stripped and OVA-treated BN rat skin. When siRNA was applied on the skin without iontophoresis (passive diffusion), Cy3-labeled siRNA (red) was observed only on the surface of the skin (Fig. 2A). In contrast, siRNA was observed inside the skin following iontophoresis (Fig. 2B). These observations suggested that the penetration of siRNA through the skin was enhanced by iontophoresis. Furthermore, siRNA was detected in the upper layer at the epidermal/dermal junction (Fig. 2B). This result indicates that siRNA delivered by iontophoresis accumulates in the epidermis, but does not pass through the basal cell layer into the dermis. The reason why siRNA concentrated exclusively in the epidermis is unclear. The result might be due to the complex structure of the basal cell layer. In any event, iontophoresis effectively delivered siRNA to target epidermal cells, including keratinocytes, and avoided the distribution of siRNA to the peripheral circulation.

Next, we asked whether siRNA delivered via iontophoresis could alter the level of expression of a target gene involved in AD. IL-10 is produced by dermal infiltrating monocytes/macrophages and Th2 lymphocytes and is one of the causes of AD (Ohmen et al., 1995; Simon et al., 2004). IL-10 is also produced by keratinocytes in response to various stimuli, such as tape stripping and allergen exposure (Nickoloff et al., 1994). Moreover, topical application of antisense oligonucleotides against IL-10 showed a therapeutic effect on AD in a murine model (Sakamoto et al., 2004). In this study, IL-10 was selected as a target endogenous gene to determine the efficacy of siRNA. IL-10 mRNA was hardly detected in the skin of the non-treated group (Fig. 3). In contrast, significant expression of IL-10 in the skin was induced by tape stripping and OVA treatment. As anticipated, the level of IL-10 was not significantly different in the negative control animals that received anti-luciferase siRNA treatment (Fig. 3). Importantly, the level of IL-10 transcripts in OVA-treated skin was significantly reduced (73 ± 7%, p = 0.024) by iontophoresis of anti-IL-10 siRNA as compared to the OVA-treated group (Fig. 3). Therefore, the elevated IL-10 mRNA induced under AD-like conditions was suppressed by RNAi when anti-IL-10 siRNA was delivered by iontophoresis. A 73% reduction of IL-10 mRNA level by iontophoresis of siRNA might be significant for the treatment of AD skin lesions, since a therapeutic effect on AD in a murine model was observed when that level was reduced (70%) by topical application of antisense oligonucleotides targeted against IL-10 (Sakamoto et al., 2004). Our results were achieved in the absence of any transfection reagents, consistent with a previous report using intradermal injection of siRNA (Wang et al., 2007a,b).

Topical introduction of siRNA into the skin by intradermal injection (Wang et al., 2007a,b) or electroporation (Inoue et al., 2007) had been performed at a dosage of 20 μg or 12.5 μg, respectively. Moreover, topical application of antisense oligonucleotides against IL-10 has been done at 120 μg (Sakamoto et al., 2004). In this study, iontophoretic siRNA delivery was performed at a dosage of 10 μg. After 1 h of iontophoresis, approximately 50% of the dose remained in the absorbent cotton patch (data not shown). Therefore, the significant reduction of targeted gene expression was actually achieved with less siRNA delivered into the skin than that previously described. If the release efficacy could be improved, our system may be even more useful for the delivery of siRNA into the skin. The effect of RNAi persisted for 12 h, but not 24 h (data not shown), after iontophoresis. This might be attributed to the limited stability of siRNA in the skin. Therefore, it is also necessary to improve the stability of siRNA in this setting.

The iontophoretic siRNA delivery system is superior to intradermal injection of siRNA or topical application of antisense oligonucleotides because no tissue damage resulted from the application of iontophoresis with constant current density, confirming our previous study (Kigasawa et al., 2009). Furthermore, there was no alteration in the level of transcripts for house keeping genes such as β-actin (data not shown). This suggests that there were minimal nonspecific effects. Note that the suppression of targeted gene mRNA was achieved at a low dosage of siRNA. Moreover, siRNA specifically accumulated in the epidermis during iontophoresis. Delivering siRNA only to the epidermis by intradermal injection...
is difficult. Finally, the iontophoretic delivery system can be readily adapted to larger affected areas of the skin, because delivery efficacy depends on the current value per unit area (Kalia et al., 2004).

In conclusion, we report the development of an iontophoretic siRNA delivery system specific for the epidermis. Significant silencing of the target gene, IL-10, was achieved. This is the first report to demonstrate that siRNA delivered by iontophoresis specifically accumulates in the viable epidermis and that transcription of an endogenous cytokine can be blocked. Thus, this siRNA delivery system is a promising method for the therapy of skin diseases.
Fig. 3. Effects of OVA and siRNA treatments on IL-10 transcript levels in BN rat skin. IL-10 transcript levels in the skin were determined by semi-quantitative RT-PCR and PCR products were subjected to gel electrophoresis (A). (a) Non-treated BN rat skin; (b) OVA-treated BN rat skin; (c) OVA and anti-luciferase siRNA treated BN rat skin; (d) OVA and anti-IL-10 siRNA treated BN rat skin. Band intensities were analyzed with the CS analyzer 2.0. (B) The graph represents the relative IL-10 transcript levels corrected by 18S rRNA as an internal standard and results are shown as means ± SD for four rats in each group. The statistical significance of mean comparisons was determined by Student’s t-test. * p < 0.05.

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