Anti-Inflammatory Effects of Potassium Iodide on SDS-Induced Murine Skin Inflammation

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Potassium iodide (KI), initially derived from seaweed in the early 19th century, is used for treating sporotrichosis in dermatological practice. KI has also been used to treat several noninfectious inflammatory skin diseases. However, the mechanisms underlying the improvement in such skin diseases remain unknown, and KI is not used widely. Thus, although KI is an old drug, physicians may not prescribe it frequently because they lack knowledge about it. Although KI is very inexpensive and causes few side effects, it has been superseded by new powerful and expensive drugs, such as biological agents. We applied 3% KI topically to areas of inflammation in mice. The levels of IL-1β and TNF-α gene expression were reduced, whereas that of IL-10 gene expression was increased. Small interfering RNA that was designed to reduce IL-10 gene expression levels was injected into the same mice, and the anti-inflammatory effects of KI were not observed. Thus, the pharmacological action of KI is based on its anti-inflammatory effects caused by the increase in IL-10 levels. This information would increase dermatologists’ awareness of KI as an efficacious and cost-effective treatment.


INTRODUCTION
Among low-molecular-weight chemical substances, some drugs, apart from their primary actions, exhibit other pharmacologic actions in vivo; for example, dimethyl fumarate, which is used in the treatment of multiple sclerosis and psoriasis, was once used as a preservative for leather products (Silvestre et al., 2010). However, it exerts anti-inflammatory effects by decreasing the phosphorylation of NF-kB and the expression of IL-6 and TNF-α (Li et al., 2017). Potassium iodide (KI), originally derived from seaweed in the early 19th century, was initially used to treat thyroid disorders. However, KI is most useful in the dermatological field for treating cutaneous sporotrichosis and erythema nodosum (Hayashi et al., 2017). In addition, KI is reportedly useful in treating several noninfectious inflammatory skin disorders, such as palmoplantar pustulosis, cutaneous manifestations of Behçet disease, Sweet syndrome, and pyoderma gangrenosum (Costa et al., 2013; Hayashi et al., 2017; Sterling and Heymann, 2000). Therefore, KI may also have some anti-inflammatory effects. Meanwhile, the mechanisms underlying the improvement in these diseases remain unknown, and the use of KI is not widespread. Thus, although KI is an old drug that is very inexpensive and causes few side effects, physicians frequently may not prescribe it because they lack knowledge about it. Currently, Behçet disease and palmoplantar pustulosis are treated with biological agents, such as anti-TNF-α antibody and anti-IL-23p19 antibody, respectively. In addition, IL-1 receptor antagonist has been reported to be effective in treating Sweet syndrome (Kluger et al., 2011) and pyoderma gangrenosum (Beynon et al., 2017). Thus, KI has been superseded by new powerful and expensive drugs, such as biological agents, in this dermatological practice.

Murakami et al. (2011) reported that patients with palmoplantar pustulosis—a common, chronic, and very refractory inflammatory skin disorder—had increased levels of IL-17–related cytokines, such as TNF-α and IL-1β, in skin lesions and serum. Hayashi et al. (2017) reported the success and effectiveness of KI in treating 25 cases of palmoplantar pustulosis; thus, we speculated that the anti-inflammatory effects of KI treatment are related to the regulation of these inflammatory cytokines. Accordingly, we studied the effect of KI specifically on neutrophils, M1 and M2 monocytes, CD4⁺ and CD8⁺ T cells, and regulatory T cells. To do so, we applied SDS epicutaneously to induce inflammatory lesions in the skin of wild-type mice. This model promotes disruption of barrier function, which then leads to an inflammatory response, marked by leukocyte invasion.

By elucidating the pharmacologic mechanism underlying the therapeutic effects of KI, we hoped to increase dermatologists’ awareness of KI as an effective treatment. If strong evidence of its efficacy were to lead to widespread use of KI in clinical practice, it could prove to be very cost effective as well.

RESULTS
Alleviating cutaneous inflammation by KI treatment
In mice that received SDS and KI, the inflammatory skin response was almost completely ablated (Figure 1a), whereas in the control mice, which received SDS and PBS, the inflammatory skin response remained (Figure 1b). In the control
group, superficial cutaneous inflammation and keratosis were reported, and the skin specimens were marked by leukocyte invasion, vasodilation, epidermal hyperproliferation, and edema (Figure 1c); these findings did not characterize mice that received SDS and KI (Figure 1d). The epidermis of mice that received SDS and KI (20.6 ± 3.0 μm) was significantly thinner than that in control mice (118.8 ± 7.0 μm) (Figure 1e).

### Effects of KI treatment on expression levels of IL-1β, IL-10, IL-17, IL-35, and TNF-α in SDS-induced inflammatory skin

Regarding TNF-α and IL-1β, the mRNA expression level in the mice treated with SDS and KI was significantly lower than that in the control mice (53.5% ± 6.4% and 67.0% ± 8.8%, respectively). Regarding the level of IL-17 and IL-35 (35bi3) gene expression, no significant difference was noted between the KI-treated and control mice (95.0% ± 8.3% and 115.5 ± 7.0%, respectively). However, the IL-10 and IL-35 (IL-12p35) gene expression levels were significantly higher in the KI-treated than in the control mice (225.8 ± 19.0% and 165.5% ± 14.4%, respectively) (Figure 2a–f). In the samples of protein obtained from the skin, the TNF-α expression level in the mice treated with SDS and KI was significantly lower (2.20 ± 0.06 pg/μg skin lysate) than that in the control mice (1.38 ± 0.06 pg/μg skin lysate), as assessed by ELISA. However, the IL-10 expression levels did not differ significantly between the KI-treated mice and control mice (1.58 ± 0.09 vs. 1.63 ± 0.13 pg/μg skin lysate, respectively) by ELISA (Supplementary Figure S1).

### KI alleviates inflammatory cell invasion in SDS-induced skin inflammation

Monocyte (CD11b+) and neutrophil (Gr-1+) counts in the KI-treated mice were significantly lower than those in the control mice; monocyte counts decreased from 11.7% ± 1.4% to 5.6% ± 0.9%, and neutrophil counts decreased from 5.5% ± 0.5% to 1.8% ± 0.3% (Figure 3a). M1 monocyte (CD80+) counts in the KI-treated mice decreased significantly (from 41.5% ± 3.2% to 12.7% ± 2.0%), whereas M2 monocyte (CD163+) counts, which were low, did not differ significantly between the KI-treated mice (1.2% ± 0.2%) and control mice (1.5% ± 0.2%) (Figure 3b). The CD8+ T-cell count in KI-treated mice decreased significantly in the control mice (from 12.5% ± 0.6% to 6.7% ± 0.4%), whereas CD4+ T-cell counts did not change significantly (from 3.7% ± 0.4% to 3.3% ± 0.3%) (Figure 3c). Regulatory T-cell (CD4+ and CD25+) counts were low and did not differ significantly between the KI-treated mice (0.6% ± 0.2%) and control mice (0.7% ± 0.1%) (Figure 3d).

### KI did not alleviate skin inflammation when IL-10 was reduced

The IL-10 knockdown condition was created by injection of small interfering RNA (siRNA). The IL-10 expression level decreased to 9.4% ± 1.2% in the mice treated with PBS after siRNA injection compared with the mice treated with PBS without siRNA injection. Dermatitis was not observed in the phenotype among these groups (Figure 4). Under the IL-10 knockdown condition, no remarkable differences were observed in the PCR assay of cytokines, histopathologic findings, and the phenotypes between the mice groups treated with PBS alone and the untreated group (only siRNA injection; data not shown). In the mice that received negative control siRNA, SDS-induced skin inflammations; however, initiating KI treatment improved the dermatitis (Figure 4). There were no significant differences between the mice without siRNA injection and those that received the negative control siRNA injection in terms of the mRNA expression levels of IL-10, IL-1β, TNF-α, and IL-35 (IL-12p35) after treatment with PBS, SDS + PBS, and SDS + KI. Only the data
between the mice treated with SDS

The neutrophil (Gr-1/C6 (0.5%) significantly higher than those in mice treated with PBS alone (0.7%) were higher than those in mice treated with PBS alone (0.5% ± 0.1%), and there was no significant difference between the mice treated with SDS + PBS and KI. The neutrophil (Gr-1+) counts in the mice treated with SDS and PBS (1.8% ± 0.2%) or KI (2.0% ± 0.5%) were higher than those in the mice treated with PBS alone (0.7% ± 0.1%), but there were no significant differences among the mice treated with SDS + PBS or KI and PBS alone (Figure 5).

Under the IL-10 knockdown condition, the mRNA expression levels of TNF-α, IL-1β, and IL-35 (12p35) in mice receiving SDS and KI increased significantly compared with those in mice treated with SDS and PBS (from 281.7% ± 39.3% to 507.0% ± 7.45%, from 219.8% ± 48.2% to 462.1% ± 30.8%, and from 202.0% ± 40.2% to 381.1% ± 21.1%, respectively). The IL-10 mRNA expression levels in the mice treated with SDS and KI increased under the IL-10 knockdown condition (from 11.2% ± 1.8% to 32.1% ± 1.3%). However, no significant difference was observed between these groups (Figure 6a–d). In the samples of protein obtained from the skin, which were assessed by ELISA, the TNF-α expression level in the mice treated with SDS and KI was significantly higher than that in the SDS and PBS-treated mice (3.71 ± 0.41 vs. 2.54 ± 0.08 pg/μg skin lysate, respectively). The IL-10 expression did not differ significantly in the mice treated with SDS and KI (0.34 ± 0.05 vs. 0.26 ± 0.05 pg/μg skin lysate, respectively) (Supplementary Figure S1).

**DISCUSSION**

Lipsker and Lenormand (2012) described excessive production of inflammatory cytokines, mainly IL-1, in neutrophilic dermatitis, such as Sweet syndrome and pyoderma gangrenosum, and they reported the efficacy of IL-1-blocking therapies. In another study, Carlos et al. (1994) reported production of IL-1 and TNF-α by adherent peritoneal cells in BALB/c mice after intravenous inoculation with the fungus *Sporothrix schenckii*. Sporotrichosis is one of the diseases best known to respond to KI (Hassan and Keen, 2012). In SDS-induced inflammation in murine skin, the expression of TNF-α or IL-1 is increased, and this mouse model has been widely used in dermatological research (Cramer and Johnson, 2003; Thepen et al., 2000). We speculated that the effect of KI, a drug with known efficacy in treating several diseases, could be easily verified with the use of a simple dermatitis model, such as this model. Clinically, KI is generally used orally; however, it was impossible to quantitively intake in mice. In addition, injection into the peritoneal cavity or vein could result in cardiac arrest because it causes a rapid increase in blood concentration of potassium. KI dissolves well in water, and so, it is suitable for the examination with topical treatment. In a human study, radioactive iodine-131 was topicaly applied during an investigation of transdermal absorption of iodine (Harrison, 1963). When a low-concentration aqueous

![Graphs and images](https://www.jidonline.org/2003)
solution of potassium and iodine-131 was applied to one forearm and the cumulative urinary radioactivity was measured as an index, the absorptivity was estimated to be approximately 0.1%. In addition, topical vitamin D3, whose molecular weight is greater than that of KI, has been reported to reach the dermis (Michel et al., 1997). Although we attempted percutaneous administration of KI in this study, iodine has an atomic mass of only 132 Da and can be expected to penetrate the epidermis.

IL-10 is known as a regulatory cytokine (Dambuza et al., 2017). When IL-10 was reduced by siRNA, the anti-inflammatory effect of KI was not observed. Instead, inflammatory cytokine levels were increased. Highly concentrated KI reportedly causes dermatitis (Massé et al., 2008), and in our experiment, when 10% KI was applied to murine skin, dermatitis was induced (Supplementary Figure S2a and b). Although KI probably increases inflammatory cytokine levels, it could have an anti-inflammatory effect at low concentrations, and the action of IL-10 might cancel out the action of KI. The results of our study reveal that an increase in IL-10 expression is one of the mechanisms by which KI exerts its anti-inflammatory effect.
effects, but which type of cell interacts with KI is still unknown. Because our study design entailed topical application, we suspected that, because of direct effects by cutaneous keratinocytes, KI will penetrate the epidermis and interact with inflammatory cells in the dermis, or the KI absorbed into the blood affects the leukocytes in the blood to produce IL-10. In this study, we focused on inflammatory cells that might be influenced by KI in the skin.

The production of IL-10 from M2 macrophages, regulatory T cells, and CD4⁺ T cells has been reported (Motomura et al., 2011). The model mice in our study produced very
few M2 macrophages and regulatory T cells, and flow cytometry analysis revealed no significant difference between the KI-treated mice and control mice. These findings indicate that CD4<sup>+</sup> T cells could produce IL-10 as a result of KI treatment. We accordingly examined a double immunostaining of IL-10 and CD4. CD4<sup>+</sup> cells showing overlapped staining with IL-10 were abundant among SDS and KI-treated mice, in comparison with SDS and PBS-treated mice (Supplementary Figure S3).

IL-35 is also known as a regulatory cytokine (Dambuza et al., 2017). IL-35 is produced in regulatory T and B cells (Li et al., 2012). The levels of CD20<sup>+</sup> cells, which are markers for all types of B cells, were quite low, as demonstrated by immunostaining (data not shown); moreover, flow cytometry analysis showed that populations of regulatory T cells were also quite low. KI causes an increase in IL-35, but it was not clear from this study which cells produced IL-35, whether KI specifically affected CD4<sup>+</sup> cells, or whether KI or PBS were higher than those in the mice treated with PBS alone, but there was no significant difference among the mice treated with SDS + PBS and/or KI and PBS only. Data are presented as mean ± SEM. *P < 0.0005. KI, potassium iodide; PE, phycoerythrin; n.s., not significant.

In conclusion, the mechanism of action by KI in alleviating skin disease is its anti-inflammatory effect, which results from the increase in IL-10 and IL-35 levels. Moreover, the effect of KI on CD4<sup>+</sup> T cells should also be studied. Motomura et al. (2011) reported that the elevated activity of E4BP4, one transcription factor in CD4<sup>+</sup> T cells, is responsible for the regulation of IL-10 production. It is interesting to note that activation of E4BP4 by KI leads to elevation of IL-10 levels.

We believe that KI, an old and inexpensive drug, will be promoted as a treatment for inflammatory skin diseases.

**MATERIALS AND METHODS**

**Animals**
Specific pathogen-free, 6-week-old female BALB/c mice (weighing approximately 20 g) were purchased from Japan SLC (Hamamatsu, Japan) and fed with food and water ad libitum during all treatment procedures.

**Assessment of the topical application of 5% SDS and 3% KI**
A total of 5% SDS was sterilized by filtration and applied daily to the shaved skin on the backs of the mice for 7 days. During that time, four of the mice were treated with 3% KI (Nichi-Iko, Toyama City, Japan) solution in PBS (n = 4), and four other mice were treated with PBS as a control group (n = 4) (Supplementary Figure S4a). In addition, to emphasize the clear difference between treatment results, four additional mice received no treatment (n = 4). The application of a 5% SDS solution in PBS for 7 days in wild-type mice leads to an inflammatory response marked by leukocyte invasion, vasodilation, epidermal hyperproliferation, and edema (Cramer and Johnson, 2003; Thepen et al., 2000). The topical application of highly concentrated KI reportedly causes dermatitis; thus, we needed to test several different concentrations (0−10%) of KI in a preliminary study to determine an adequate concentration of KI (n = 4). The results of the preliminary study

![Image](image-url)
revealed that mice treated with 3% KI expressed the lowest amounts of TNF-α (Supplementary Figure S2a); therefore, we used 3% KI in the main study.

Assessment of siRNA injection
Under the IL-10 knockdown condition, the mice were treated with SDS and KI or PBS, PBS alone, and untreated in addition to siRNA (Stealth RNAi siRNA; Thermo Fisher Scientific, Waltham, MA) (Wu et al., 2019). The RNA sequence, as shown in Supplementary Table S1, was designed and administered by means of injection to knock down the effects of IL-10 within 10 days. Two days before the application of SDS and PBS or KI and PBS alone, siRNA with a transfection reagent (Invivofectamine 3.0 Reagent; Thermo Fisher Scientific) was injected into the caudal veins of the mice (Supplementary Figure S4b), according to the manufacturer’s protocol. To verify the effects of IL-10 knockdown by siRNA and noninterference against other cytokines, mice that received negative control siRNA (Stealth RNA siRNA Negative Control Med GC Duplex #2; Thermo Fisher Scientific) were used. Under the IL-10 knockdown condition, the PCR assays of mRNA of the six groups were compared (n = 4), which included treatment with PBS alone and SDS and PBS or KI, with or without the siRNA injection, and the control group received treatment with PBS alone without the siRNA injection.

Statistical analysis
The differences between the two groups (Figures 1 and 3) were analyzed using Student’s t-test. For the differences between more than two groups, Tukey’s test (multiple comparisons) was used for statistical analysis. P-values of 0.05 or lower were considered significant. The data are presented as the mean ± SEM. Statistical comparisons were performed using SPSS version 18 (SPSS, Inc, Chicago, IL).

Approval of animal studies
Animal studies were approved by the review board of Dokkyo Medical University, Tochigi, Japan (approval no. 939, 2016-2020).

Data availability statement
All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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CONFLICT OF INTEREST
The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS
Conceptualization: SH, MK, IK; Data Curation: SH, SI, TS, GK; Formal Analysis: EI, TS, GK; Funding Acquisition: SH, MK; Investigation: SH, TK; Project Administration: SH; Supervision: YH, IK; Writing - Original Draft Preparation: SH

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2020.01.028.

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