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ARTICLE

Comprehensive, Multimodal Characterization of an Imiquimod-Induced Human Skin Inflammation Model for Drug Development

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Imiquimod (IMQ) is often used as a topical challenge agent to provoke local skin inflammation. The objective of this study was to develop and refine a rapid, temporary, and reversible human skin inflammation model with IMQ for application in clinical drug development. A randomized, vehicle-controlled, open-label, dose-ranging study was conducted in 16 healthy male subjects. IMQ (5 mg) was applied once daily for 72 hours under occlusion to intact skin ($n = 8$) or tape stripped (TS) skin ($n = 8$). Although IMQ alone induced limited effects, TS+IMQ treatment showed larger responses in several domains, including erythema and perfusion ($P < 0.0001$), mRNA expression of inflammatory markers ($P < 0.01$), and inflammatory cell influx compared with vehicle. In conclusion, a rapid, human IMQ skin inflammation challenge model was successfully developed with a clear benefit of TS prior to IMQ application. Future interaction studies will enable proof-of-pharmacology of novel compounds targeting the innate immune system.
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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
✔ Murine skin inflammation models with imiquimod are widely implemented to study skin inflammation. No healthy volunteer models for the application in drug development programs were explored before.

WHAT QUESTION DID THIS STUDY ADDRESS?
✔ What is the most suitable skin inflammation model with imiquimod in healthy volunteers for drug development programs?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
✔ Imiquimod application with tape stripping prior to the first dose administration is a suitable model to quickly induce temporary skin inflammation in healthy volunteers.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
✔ Future interaction studies with the model will enable proof-of-pharmacology of novel compounds targeting the innate immune system in healthy volunteers.

Skin inflammation is a physiological immune response to various stimuli including skin trauma, physical challenge, and exposure to xenobiotics, microbes, and parasites. Dysregulation of this immune response is involved in chronic inflammatory skin diseases, e.g., psoriasis vulgaris, acne vulgaris, and atopic dermatitis.[1,2] Although much mechanistic insight has been gained, including involvement of the innate immune system via Toll-like receptors (TLRs) and the adaptive immune system, the pathophysiology of skin inflammation is complex and remains to be elucidated further.[3]

Different models that mirror aspects of chronic inflammation have been developed to study skin inflammation. For instance, rapid, acute skin inflammation can be induced by topical, cutaneous application of imiquimod cream (IMQ; Aldara). IMQ application leads to agonistic activation of TLR7- and TLR8-mediated MyD88 signaling, activation of nuclear factor kappa B (NF-κB), and the induction and release of proinflammatory cytokines, type-1 interferons, chemokines, and other mediators. Ultimately, this leads to an innate and Th1- and Th17-weighted cellular immune activation and enhancement of proinflammatory effects.[4]

Although the use of IMQ appears to be safe and reasonably tolerated, disease exacerbation can occur in psoriasis patients and even the development of psoriasis in individuals without a prior history of the disease is reported.[5–10] Based on the initial findings, the first IMQ-induced skin inflammation mouse model was successfully developed.[11] This model has become widely accepted for preclinical studies of psoriasis because of its straightforward approach, inexpensiveness, and the fast acute inflammatory response.[11,12] Nevertheless, the murine model has some crucial disadvantages, the major ones being immediate systemic effects and the

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limited extrapolation of murine findings to humans due to differences in the immunology and TLRs.[12,13]

Therefore, several skin inflammation models with IMQ have been evaluated in humans. A short, 7-day model in psoriasis patients using IMQ next to tape stripping (TS) showed that psoriasis-like skin inflammation occurred but typical psoriasis did not develop.[5] Contact dermatitis driven by plasmacytoid dendritic cells (pDC) was observed in an extended model of 4 weeks, both in healthy volunteers and in patients with inflammatory skin diseases. Only limited aspects of the molecular signature of psoriasis were observed.[14] Other studies in healthy volunteers characterized the model by either solely focusing on biopsy biomarkers or only systemic effects after high topical doses of IMQ.[15,16] Acute and rapid IMQ-induced inflammation models in healthy volunteers with detailed characterization have not yet been reported. A well-characterized, comprehensive human model to study skin inflammation would open opportunities for understanding the pathogenesis of several skin diseases and for the profiling of novel drugs in development.

With this study we aimed to develop a skin inflammation challenge model with i) topical IMQ application for 24, 48, and 72 hours on a fully competent skin barrier, and ii) topical IMQ application for 24, 48, and 72 hours on TS-perturbed skin barrier to enhance drug delivery. Cetomacrogol cream, an indifferent neutral emollient, was used as the control. Skin inflammation was assessed by measurement of erythema, perfusion, and using biopsy material (mRNA expression, histology, immunohistochemistry). In the future, these models may be used in drug development programs for proof-of-pharmacology, drug profiling, or interaction studies of novel compounds targeting the innate immune system and translational research of inflammatory skin diseases.

METHODS

The protocol of this randomized, open-label, vehicle-controlled, parallel-cohort, dose-ranging study was approved by the independent Medical Ethics Committee "Medisch Ethische Toetsingscommissie van de Stichting Beoordeling Ethiek Biomedisch Onderzoek" (Assen, The Netherlands). The study was conducted according to the Dutch Act on Medical Research involving Human Subjects (WMO). Subjects were recruited throughout the Netherlands via advertisement campaigns on the Internet and in the newspaper. All subjects gave informed consent prior to any study procedure. The study was conducted from May 2016 to June 2016 at the Centre for Human Drug Research, Leiden, The Netherlands.

Study population

Sixteen[16] healthy male Caucasian (Fitzpatrick skin type I–II) volunteers, aged 18–45 years, were included in the study. Health status was verified with a medical history, physical examination, laboratory tests, and 12-lead electrocardiodiagrams (ECG). Subjects with a medical history or family history of psoriasis or any disease associated with immune system impairment were excluded from the study. Previous use of imiquimod, resiquimod, or gardiquimod was not allowed.

IMQ treatment

Prior to the first dose administration, four treatment areas (squares) were identified on the upper back and marked with a dermatological marker. During the treatment period a standard daily dosage containing 5 mg IMQ (100 mg Aldara) was applied under occlusion by a 12-mm Finn chamber (Smart Practice, Phoenix, AZ), meaning the Finn chamber was replaced with a new dose after 24 hours. Treatment area 1 was treated 1 day (5 mg IMQ, 24 hours), treatment area 2 was treated 2 days (cumulative 10 mg IMQ, 48 hours), and treatment area 3 was treated for 3 days (cumulative 15 mg IMQ, 72 hours). Treatment area 4 was treated with 100 mg cetomacrogol (indifferent) cream for 72 hours (negative control) (**Figure 1a**). The sample size and dose were selected based on a previous study with imiquimod in healthy volunteers. Given the exploratory character of the study, no formal power calculation was performed. Subjects were randomized 1:1 to receive these treatments either over a fully competent skin barrier or over a disrupted skin barrier by TS of the skin. Tape stripping enhances drug delivery over the skin barrier. It is known from the literature that only a limited amount of drug can be delivered over a fully competent barrier. Given the short treatment duration in this study it was decided to tape strip one group before application to ensure drug delivery. The TS procedure was performed as follows: tape (D-Squame, CuDerm, Dallas, TX) was applied to the marked treatment area and a roller was used to press the tape to the skin to avoid furrows and wrinkles by a single operator. After this the tape was removed at a constant velocity. The procedure was repeated for at least 15 times until the transepidermal water loss (TEWL) by Aquaflux (BioX, London, UK) was between 20–25 g/m^{2}h (partial removal of the stratum corneum). Safety and tolerability were monitored by recording adverse events.

Clinical scores

Clinical assessments were performed daily of treatment area 3 and 4, and included visual erythema grading (Clinician Erythema Assessment (CEA) scale, 0 represents absence of erythema, 4 very severe), colorimetry (a* value (DSM II ColorMeter, Cortex Technology, Hadslund, Denmark) with a total of three repeats on each treatment area, 2D photography erythema index analysis, and perfusion by laser speckle contrast imaging (LSCI; PeriCam PSI System, Perimed Jäfälla, Sweden).

Transdermal analysis patch (TAP)

Skin surface biomarkers were collected predose and after 1, 2, and 3 days of treatment by with TAP (FibroTx, Estonia). TAP consists of a multiplex capture-antibody microarray that is supported by a dermal adhesive bandage for fixture to skin and can measure up to six markers per TAP. IFN-β, IFN-γ, Il-8, IL-6, HBD-2, IL-1b were chosen and captured from skin and were qualitatively and quantitatively analyzed by spot-ELISA (enzyme-linked immunosorbent assay).

Skin punch biopsies

Three millimeter skin biopsies were collected predose (of tape stripped skin in the TS cohort), 24 hours after end-of-treatment of each treatment area, and from a distant site with

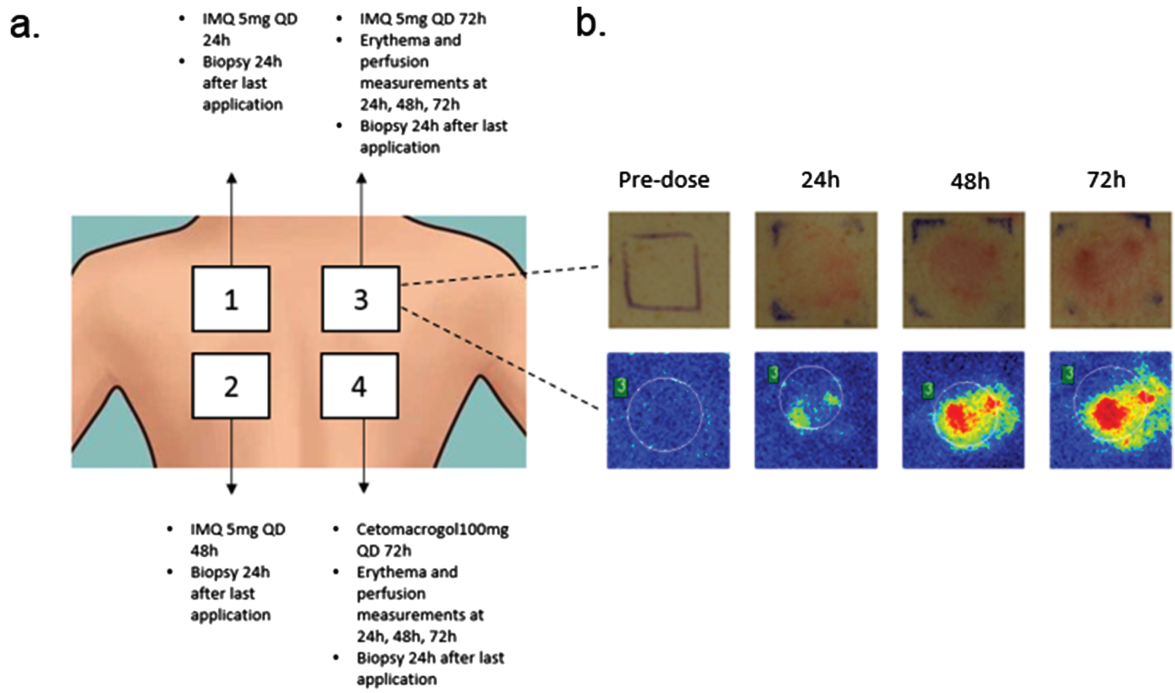

Figure 1 (**a**) Treatment schedule of the study. Treatment areas 1 and 2 served as biopsy sites for biopsies after 1 (24 hours) and 2 (48 hours) days of treatment. Treatment sites 3 and 4 served as sites for all erythema and perfusion measurements and biopsies after the longest treatment duration (3 days, 72 hours, 5 mg imiquimod (IMQ) application at 0 hour, 24 hours, and 48 hours). In eight subjects the local treatment area was tape stripped before IMQ application. All treatments were applied under occlusion by a 12-mm Finn chamber. (**b**) Clinical impression of site 3, tape stripping (TS)+IMQ 72 hours. An increase of erythema and perfusion is observed.

a total of six biopsies per subject. After harvest, the biopsies were placed in RNAlater medium and stored at 4°C. The biopsy samples were analyzed at the Immunology Laboratory at Erasmus MC, Rotterdam, The Netherlands. RNA extraction and real-time quantitative polymerase chain reaction (PCR) analysis was performed for the following biomarkers: IP10/CXCL10, IFN-β, IFN-γ, TNF-α, IL-1β, IL-6, HBD-2, MX1, MXA, and ICAM-1, which were chosen based on the murine model. Additionally, a histopathological score was obtained of hematoxylin and eosin (H&E)-stained tissue by two blinded persons for the following characteristics of psoriasis and dermatitis: general infiltration (all types of inflammatory cells present), parakeratosis, acanthosis, papillomatosis, and spongiosis. The histopathological score for each characteristic was graded based on the fold increase or decrease compared with a reference biopsy of a healthy subject not related to the clinical trial (1; equal to the reference biopsy, 2; twofold increase compared with the reference biopsy, etc.). Furthermore, immunohistochemical staining was performed to obtain scoring of markers CD11c, CD14, CD1a, CD4, CD8, and HLA-DR. This was also performed by two blinded persons and graded the same way as for the histopathological characteristics.

Statistics

All calculations were performed using SAS for windows v. 9.4 (SAS Institute, Cary, NC). Treatment effects were analyzed with a mixed model analysis of variance with fixed factors treatment, cohort, time, treatment by cohort, treatment by time, cohort by time and treatment by cohort by time, random factors subject, subject by treatment and subject by time, and the baseline measurement as covariate. To determine the differences among the treatments, contrasts on all measurements were calculated. Analysis results per variable were generated with estimates of the difference of the different contrasts and a back-transformed estimate of the difference in percentage for log-transformed parameters, 95% confidence intervals (CI) (in percentage for log-transformed parameters), and Least Square Means (geometric means for log-transformed parameters), and the *P*-value of the contrasts.

RESULTS

In total, 35 subjects were screened, of whom 26 subjects were eligible for participation in the study. All of the 16 included subjects completed the study and the treatments of IMQ or TS+IMQ were administered as depicted in **Figure 1a**. The study participants were all Caucasian and had a mean age of 22.3 (18–33) years. The treatments were in general well tolerated. The most frequent occurring treatment-emergent adverse event was application site pruritus, observed in 25% of the participants of both IMQ treatments. No increase in IFN-α or IFN-γ was detected in the systemic circulation.

IMQ application induced reversible erythematous hyperperfused skin lesions

A clear exposure-dependent increase in erythema was observed with IMQ and TS+IMQ for all erythema measurements compared with the control as determined by erythema

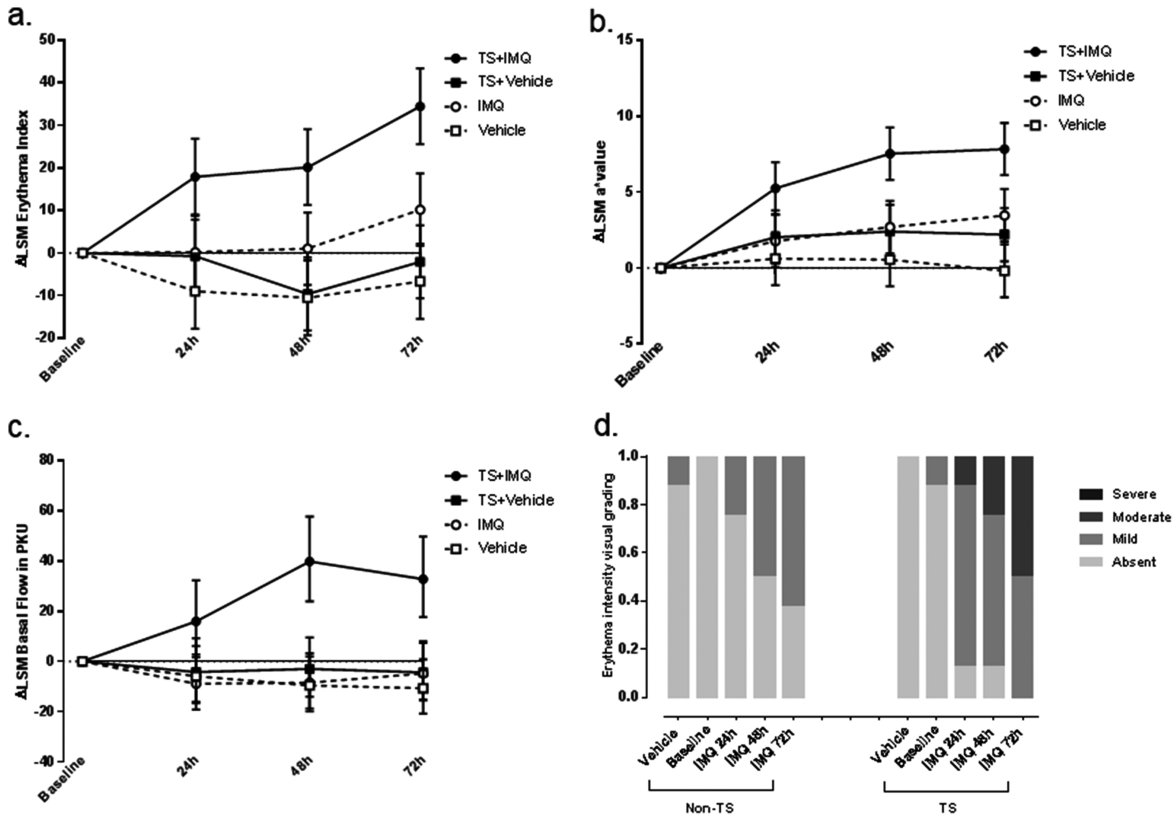

Figure 2 Erythema and perfusion induced by IMQ and TS+IMQ application. Error bars are defined as LSM ±upper and lower limit. (**a**) LSM change from baseline in erythema index, (**b**) LSM change from baseline in erythema by colorimetry, (**c**) LSM change from baseline in % by laser speckle contrast imaging, (**d**) erythema by visual grading displayed as % presence per timepoint.

Table 1 Analysis results of erythema and perfusion measurements

	IMQ vs. vehicle			TS + IMQ vs. TS + vehicle		
	24 hours	**48 hours**	**72 hours**	**24 hours**	**48 hours**	**72 hours**
Erythema index	9.18	11.55	16.84	18.64	29.76	36.53
	(–1.37, 19.73)	(1.00, 22.10)	(6.29, 27.38)	(7.89, 29.38)	(19.01, 40.50)	(25.78, 47.28)
	$P = 0.09$	$P = 0.03$	$P = 0.003$	$P = 0.001$	$P = <.0001$	$P = <.0001$
Colorimetry	1.16	2.16	3.66	3.22	5.13	5.64
	(–0.34, 2.66)	(0.66, 3.65)	(2.16, 5.16)	(1.72, 4.73)	(3.6, 6.64)	(4.13, 7.15)
	$P = 0.12$	$P = 0.006$	$P = <.0001$	$P = 0.0001$	$P = <.0001$	$P = <.0001$
Basal Flow (% change)	–3.1%	1.2%	6.6%	21.0%	44.0%	38.8%
	(–13.5%, 8.5%)	(–9.6%, 13.4%)	(–4.9%, 19.4%)	(7.0%, 36.9%)	(28.5%, 61.4%)	(23.8%, 55.5%)
	$P = 0.57$	$P = 0.83$	$P = 0.26$	$P = 0.003$	$P = < 0.0001$	$P = < 0.0001$

The differences between imiquimod (IMQ) vs. vehicle and tape stripping (TS)+IMQ vs. TS+vehicle are shown. Data are presented as mean, 95% confidence interval, and *P*-value.

index (EI) photo analysis, erythema by colorimetry, and erythema by visual grading (**Figure 1b**, **Figure 2**). Upon 48 hours IMQ treatment, the difference with vehicle was statistically significant ($P < 0.05$) (EI; 11.55, 95% CI 1.00–22.10, $P = 0.03$; colorimetry; 2.16, 95% CI 0.66 – 3.65, $P = 0.006$, **Table 1**). TS+IMQ resulted in more significant contrasts ($P < 0.01$) compared with vehicle and these were already achieved after 24 hours (EI; 18.64, 95% CI 7.89–29.38, $P = 0.001$, colorimetry; 3.22, 95% CI 1.72 – 4.73, $P = 0.0001$, **Table 1**). An exposure-dependent increase in perfusion that plateaued after 48 hours was only observed with TS+IMQ (**Figure 2c**). Concordant with the erythema, this increase was already statistically significant 24 hours after application (21%, 95% CI 7.0–36.9%, $P = 0.003$, **Table 1**). TS itself did not induce significant changes in erythema and perfusion (**Supporting Figure S1**). The skin clinically recovered after end of treatment (not shown).

IMQ-induced activation of innate immune system

Expression of CXCL10, hBD-2, ICAM-1, IFN-β, IFN-γ, IL-1β, IL-6, MX-1, MX-A, and TNF-α in punch biopsies was investigated by real-time quantitative (q)PCR analysis, and normalized for the housekeeping gene ABL. As shown in **Figure 3**, CXCL10, MX-A, ICAM-1, and hBD-2 showed a

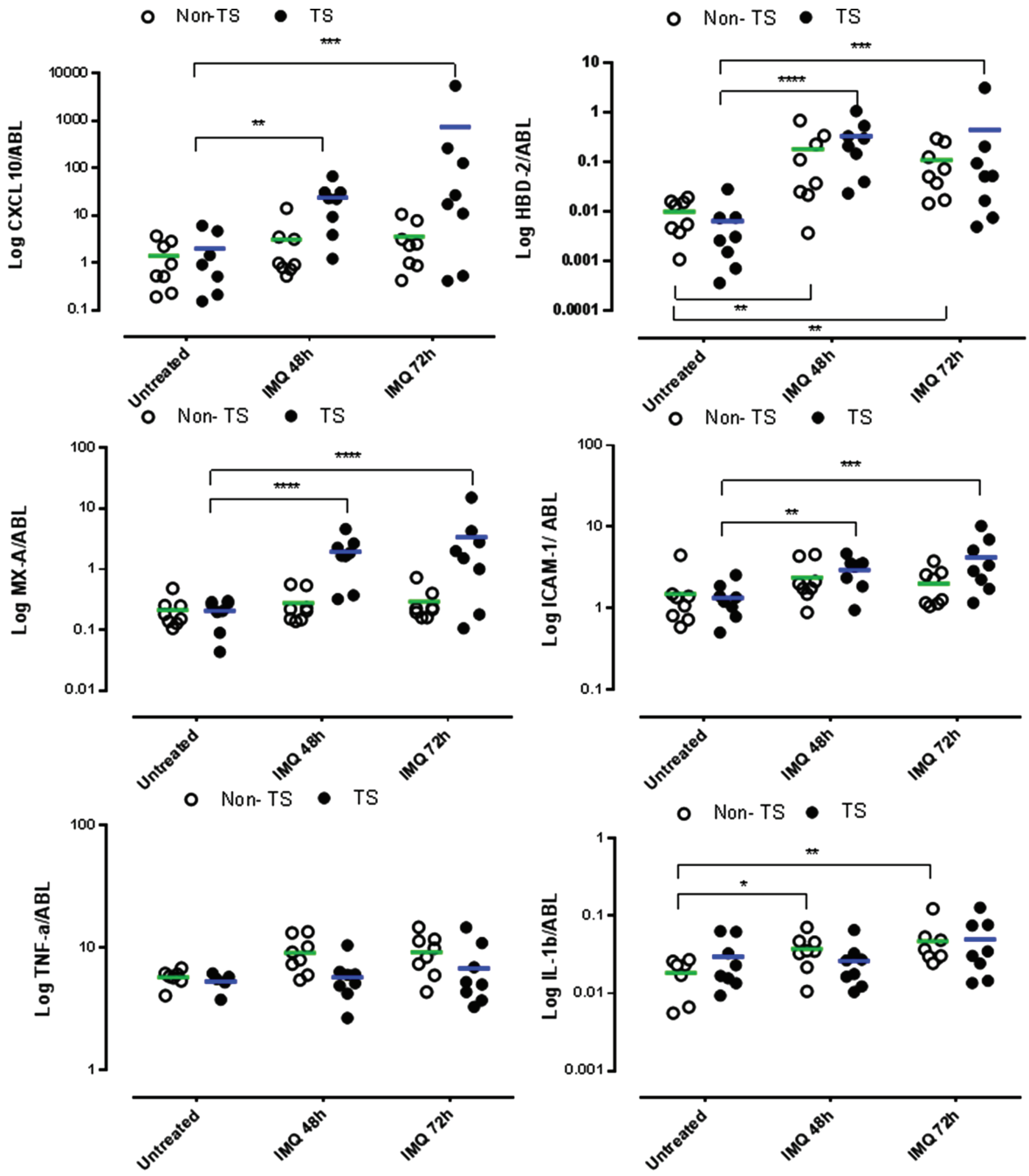

Figure 3 mRNA expression in skin over time in IMQ and TS+IMQ treated skin of CXCL10, HBD-2, MXA, ICAM1, TNF-α and IL-1β. Statistical significance is indicated as follows: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

statistically significant increase after 48 hours and 72 hours in the TS+IMQ treatment group compared with the untreated area ($P < 0.01$). This was only observed for hBD-2 in the IMQ treatment group vs. untreated. In addition, an increased expression of both TNF-α and IL-1β was observed with 48 hours and 72 hours treatment, compared with the untreated site; this was significant for IL-1β ($P < 0.05$), and only observed for the conditions without TS (**Figure 3**). TAP data were highly variable and not significantly different from untreated skin (data not shown).

Infiltration and acanthosis induced by IMQ treatment

H&E-stained skin punch biopsies were independently analyzed by two investigators blinded to treatment. In the IMQ treatment group, no changes compared with the reference biopsy were observed. TS+IMQ treatment demonstrated ≥twofold increase in general infiltration in 31% and 44% of the subjects after 48 hours and 72 hours, respectively, compared with the reference. Acanthosis as compared with the reference was two times more frequent in 19% of these subjects after 48 hours (**Figure 3**, **Figure 4**). Moreover,

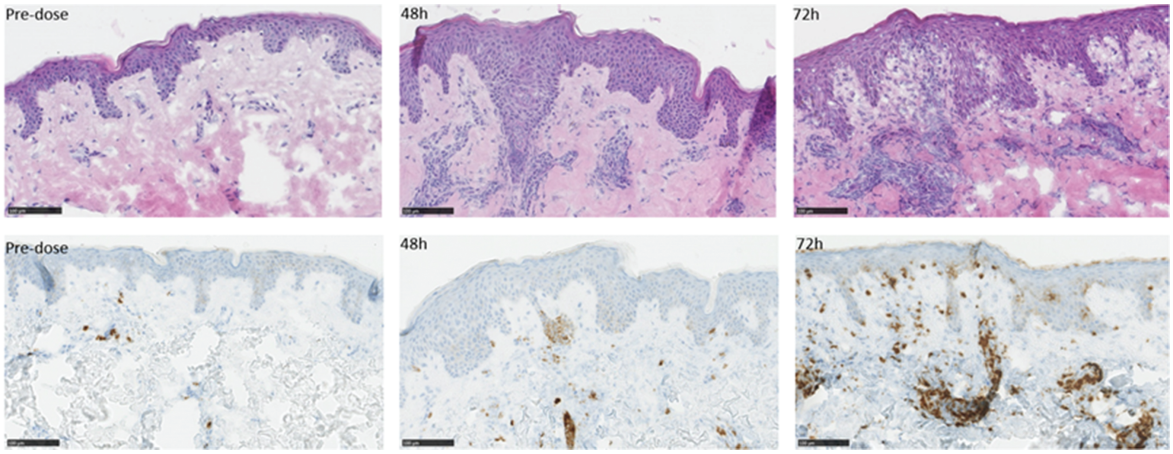

Figure 4 H&E (upper row) and CD8+ staining (lower row) over time of subject 15 treated with TS+IMQ. Scale bars = 100 μm.

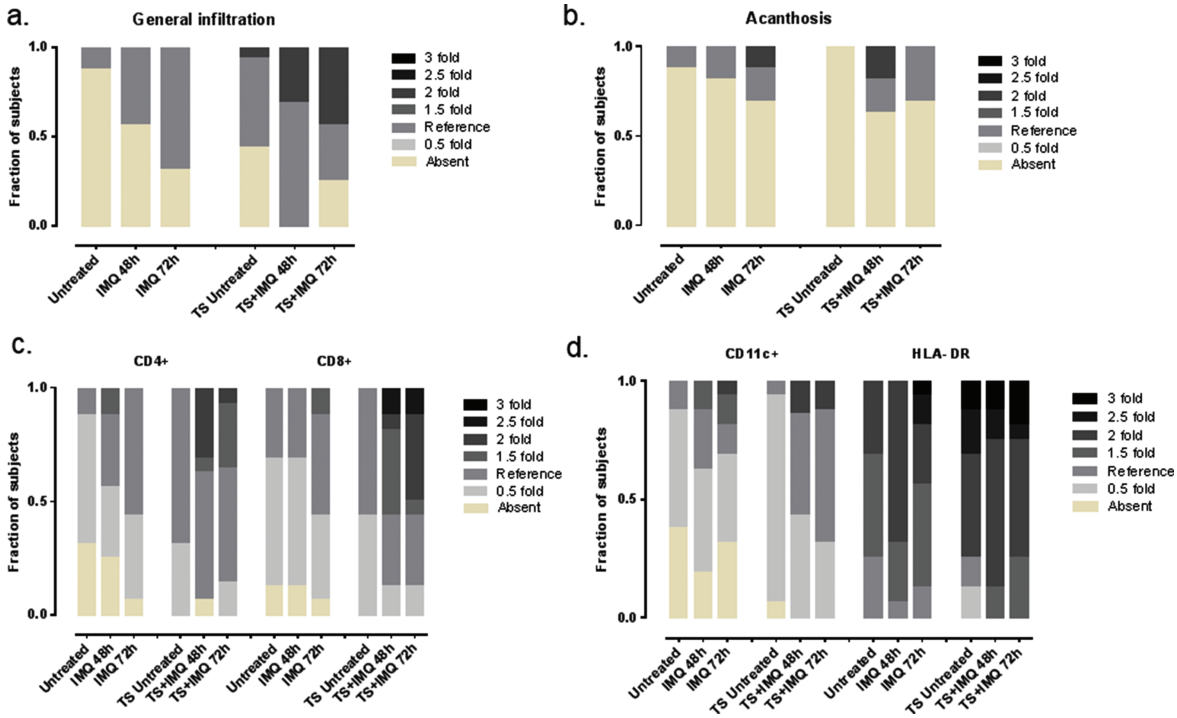

Figure 5 Histology and immunohistochemistry in skin punch biopsies of IMQ and TS+IMQ treated skin compared with untreated skin, displayed in % fold increase compared with the reference biopsy. (**a**) general infiltration, (**b**) acanthosis, (**c**) CD4+ and CD8+ infiltration, (**d**) CD11c+ and HLA-DR infiltration. Fraction of subjects is depicted.

parakeratosis, papillomatosis, and spongiosis were scored. These parameters were not different from the reference biopsy in either cohort. No histological skin changes were noted in the vehicle controls.

CD4+, CD8+, CD11+, and HLA-DR+ cells infiltrated the dermis following IMQ treatment

Immunohistochemical staining was performed to further explore cell infiltration and showed an infiltration of CD4+ T-cells, CD8+ T-cells, CD11+ dendritic cells, and HLA-DR+ macrophages, mostly in the TS+IMQ treatment group. CD8+ cells were ≥1.5 times more present in 56% of the subjects in the TS+IMQ treatment group after 48 hours and 72 hours, compared with the reference biopsy (**Figure 4**, **Figure 5**). In 37.5% of the subjects, CD4+ cells were ≥1.5 times more present examined after 48 hours and 72 hours of treatment in the TS+IMQ treatment group. Moreover, CD11c+ infiltration was slightly apparent (two times more present in 21.4% of the subjects after 48 hours, 12.5% of the subjects after 72 hours in TS). HLA-DR+ cells 12.5% of the TS subjects developed a threefold increase of HLA-DR+ cells after 48 hours of IMQ treatment, while 18.8% was observed after 72 hours of IMQ treatment, compared with the reference biopsy. However, HLA-DR was already more present at baseline in this group of subjects, compared with the reference biopsy (**Figure 5**).

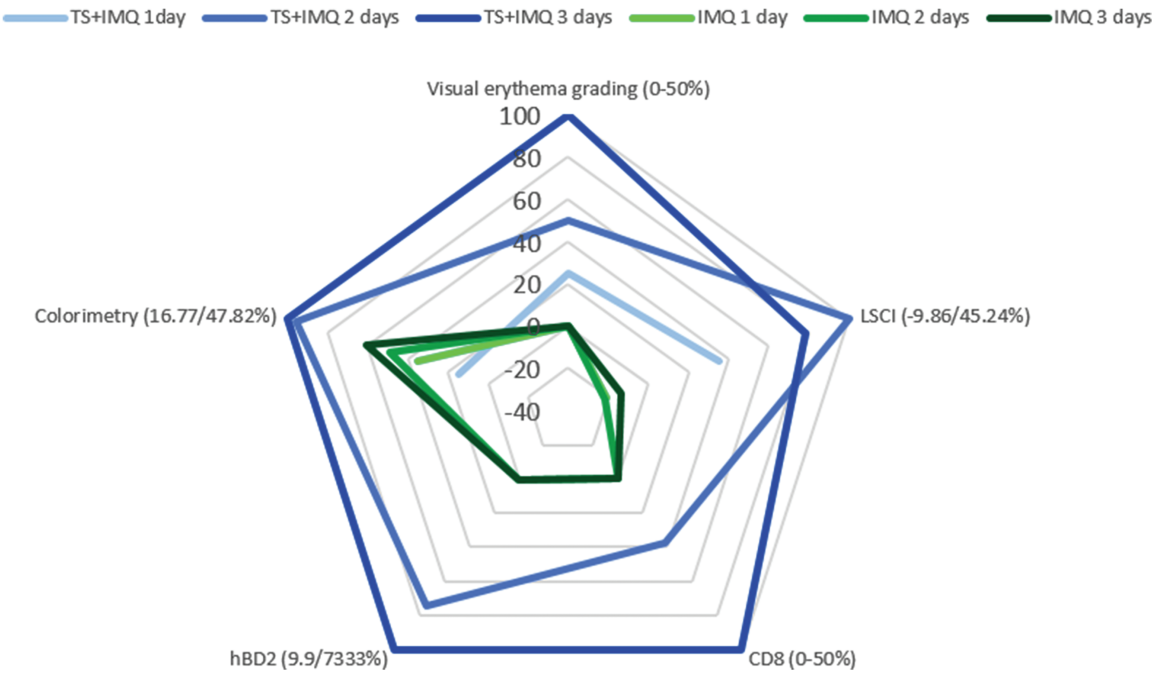

Figure 6 Multimodal assessment of the model including clinical (physician scoring), biophysical (laser speckle contrast imaging), imaging (colorimetry), molecular (mRNA expression, hBD2), and cellular (IHC, CD8+) aspects. The observed maximal effect is used for normalization of the respective axes. For the IMQ alone group, only increased erythema by colorimetry is observed without a dose-dependent relationship. In the TS+IMQ group it is clearly visible that the effects on all domains spread over the spiderplot in a dose-dependent manner.

DISCUSSION

Comprehensive characterization of the skin inflammation model

This is the first study that comprehensively characterized an acute inflammatory model combining IMQ and TS in healthy volunteers with the purpose to apply it in drug development programs. We utilized different, complementary assessment modalites to monitor the effects thouroughly, including clinical (physician scoring), biophysical (LSCI), imaging (colorimetry), molecular (mRNA expression), and cellular (IHC) aspects. The synthesis of this multimodal assessment is presented in **Figure 6** and clearly shows concordant effects on complementary modalities and a clear dose-dependency, exposure–response relationship. While previously models were developed in psoriasis patients or with a lengthy treatment period of 28 days in healthy volunteers without TS,[5,14] our study shows a rapid and reproducible way of inducing short-term inflammatory skin lesions with effects on all the different domains, as discussed in detail in the next paragraphs.

Strong agreement of measurements assessing the clinical phenotype

IMQ induced a dose-dependent increase in erythema in all measurements, which occured much quicker and more pronouced when combined with TS. Statistically significant effects on EI, colorimetry, and LSCI were already observed 24 hours after TS+IMQ treatment, vs. 48 hours without TS. There were no clear differences in erythema intensity between 48 hours and 72 hours of treatment for the TS+IMQ treatment, which is also seen in the murine model.[11] Increased skin perfusion as a result of IMQ application was only observed when it was combined with TS, and also no clear differences between 48 hours and 72 hours appeared. In a recent study where a human model with 4 weeks of IMQ treatment was developed to study psoriasis, maximal effects occurred at day 4 or later, but TS of the skin was not performed to enhance drug delivery of IMQ.[14] Importantly, in our study we showed that within 24–48 hours of the last dose, the skin fully recovered clinically (data not shown), which is in agreement with previous reports.[5] The similar clinical observations with different methods and reversibility of effects are two strong points of our approach.

Fully comprehensive mechanistic insights of molecular patterns

CXCL10, MX-A, ICAM-1, and hBD-2 were all statistically significantly upregulated in the skin of subjects treated with IMQ for 48 hours and 72 hours in combination with TS and to a lesser extent in the non-TS cohort (only for hBD-2) compared with vehicle, which is concordant with the molecular findings of Dickson *et al.*[15] This reflects the intermediate phase response of IMQ (24–72 hours) where activation of the innate as well as adaptive immune system occurs, featuring infiltration of neutrophils, lymphocytes, and macrophages, as described in a recent review of the murine translational IMQ skin inflammation models.[17] CXCL10, a chemokine that is highly expressed when keratinocytes are activated in inflamed skin, is regulated by T cells and found in psoriasis and other autoimmune diseases, which corresponds with the findings in our clinically induced skin inflammation.[1,15,18] Upregulation of

MX-A, a downstream mediator of interferons, reflects the activation of plasmacytoid dendritic cells (pDCs), which play a major role in the pathophysiology of psoriasis.[1,19,20] Furthermore, this refects the antiviral response by IMQ, which was expected since IMQ is effective against several HPV-induced skin diseases.[21,22] The mRNA expression of adhesion molecule ICAM-1 was observed to be upregulated, which corroborates previous findings showing induction by TNF-α.[23] ICAM-1 facilitates leukocyte endothelial transmigration and enhancement of skin inflammation. Upon IMQ treatment alone, no statistically significant differences in upregulation were observed between 48 hours and 72 hours in the biopsy markers, which confirms the better suitability of the TS+IMQ combination for further application in drug development.

Interestingly, in the biopsies of the non-TS cohort, we found an upregulation of TNF- α and IL-1β compared with the untreated site, while this was not present in the TS+IMQ cohort. Presumably, the initial phase (within 24 hours), where the innate immune system is activated as a consequence of release of inflammatory mediators including IFN-α, IL-1α, IL-1β, IL-6, and TNF-α and where cellular changes such as accumulation of neutrophils and proliferation of keratinocytes occur, is at a later timepoint because of less drug delivery without TS.[17] This would also explain why TNF-α and IL-1β are not upregulated in the TS cohort after 48 hours; due to enhanced penetration by TS of the skin, the initial upregulation might already have passed. This matches with the fact that erythema was observed in the TS cohort already after 24 hours, but not in the non-TS cohort, where it appeared after 48 hours. Moreover, the presence of ICAM-1 in the TS cohort confirms the presence of TNF-α in an earlier stage, and therewith the initial phase as also seen in the murine model, since it is a downstream marker of TNF-α.[23] Vinter *et al.* did still find TNF-α and IL-1β after 48 hours, but tape stripped less extensively (10 times vs. $\pm$ 15 times in our study). Hereafter, both markers also normalized while the downstream markers increased.[5]

Concordant cellular observations

Histologically, a general infiltration with little acanthosis was seen only in the TS cohort. Classical dermatitis and psoriasis characteristics such as spongiosis, acanthosis, and parakeratosis were not observed. Although some of those characteristics were reported in the literature after longer treatment, the treatment duration in this study of maximally 72 hours is presumably too short.[5,14] In psoriasis patients, exacerbations after IMQ treatment also occur only after a prolonged period of application (average 9 weeks).[7] In addition, CD11c+, HLA-DR, CD4+, and CD8+ cells infiltrated the dermis, more in the TS cohort than in the non-TS cohort and with no clear difference between 48 hours and 72 hours. CD11c+ cells reflect the inflammatory myeloid DCs. These are highly increased in the psoriatic dermis and are known to stimulate the production of type 1 helper (Th1) cytokines.[1] Likewise, the macrophages (HLA-DR) are involved in this process, which are mediated via the TLR-7 response.[24–26] Infiltration of DCs, macrophages, and T cells indicate activation of both an innate and adaptive immune response. All histological effects are consistent with the intermediate and late-phase response of IMQ. The late phase is characterized by expression of both IL-17 and IL-22 as a result of IL-23 production, and infiltration of T cells. It normally occurs after 72 hours.[17] However, due to the enhanced drug delivery by TS of the skin, this was already observed in the 48-hour biopsies.

Translational value of the inflammation model for drug development

Taken together, the IMQ-induced histological changes are highly similar to those that were observed in the murine model and have features of both psoriasis and contact dermatitis, with activation of the innate and adaptive immune system. Concordant with findings of others, no complete phenotype induction such as psoriasiform histology was observed.[5,14] However, since the purpose of the study was to develop a model for drug profiling and interaction studies, and not primarily to study disease pathophysiology, this is not considered a limitation. A limitation of the study is the open-label design, which could have led to an observer bias of the clinical erythema grading. However, since clinical scores are highly concordant with the objective erythema measures, EI and colorimetry, the bias is presumably negligible.

In conclusion, we succesfully translated the murine IMQ skin inflammation model to a fully characterized safe, rapid, and reversible human model in healthy volunteers. Clinical and histological phenotypes were fully concordant in the TS+IMQ cohort. Therefore, TS of the skin to enhance drug delivery of IMQ is required to induce a quicker and stronger inflammatory response. No significant differences in effects of IMQ were observed between 48 hours and 72 hours of application, suggesting that 48 hours of treatment is the most suitable for this model. Future interaction studies with the model in drug development programs will enable proof of pharmacology of novel compounds targeting the innate immune system.

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