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Isothiocyanates inhibit psoriasis-related proinflammatory factors in human skin

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Abstract
Objective 4-Methylthiobutylisothiocyanate (MTBI), the main rocket (*Eruca sativa*) seed isothiocyanate (ITC), and its oxidized form, sulforaphane (SFN), were assessed for their potential effects on psoriasis-related factors.
Methods MTBI and SFN were evaluated for their effect on mRNA expression and cytokine secretion in vitro in human monocytes and macrophage-like cells and ex vivo in topically treated inflamed human skin. In addition, they were assayed in vivo for morphological changes in topically treated psoriasiform human skin in severe-combined immunodeficient (SCID) mice.
Results MTBI and SFN contributed to the prevention of inflammation development and reduced ongoing inflammation by downregulating lipopolysaccharide (LPS)-induced mRNA expression of the psoriasis-related cytokines, interleukin (IL)-12/23p40 (25–58 %), tumor necrosis factor (TNF)-α (15–37 %) and IL-6 (25–71 %), in human macrophage-like cells. In monocytes, they tended to act additively on cytokine mRNA and reduced IL-12/23p40 (51 %) secretion. In an ex-vivo inflamed human skin organ culture, MTBI (1 μg/ml) reduced the secretion of IL-1 (39 %) and IL-6 (32 %). Moreover, 2/8 and 3/8 of the MTBI- and SFN-treated psoriasiform SCID mice, respectively, recovered partially or entirely from the psoriasiform process.
Conclusions Results from these models indicate the potential of rocket seed ITCs as biological agents in the therapy of psoriasis and inflammation-related skin diseases.

Keywords Isothiocyanates · 4-Methylthiobutylisothiocyanate · Sulforaphane · Skin inflammation · Psoriasis

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Abbreviations

AMV-RT	Avian myeloblastosis virus-reverse transcriptase
BITC	Benzyl isothiocyanate
COX	Cyclooxygenase
DMEM	Dulbecco's modified Eagle medium
DMSO	Dimethyl sulfoxide
dNTP	Deoxynucleotides
ELISA	Enzyme-linked immunosorbent assay
ICAM	Intercellular adhesion molecule
IL	Interleukin
iNOS	Inducible nitric oxide synthase
ITC	Isothiocyanate
Keap1/Nrf2	Kelch-like ECH-associated protein 1/nuclear factor-like 2
LPS	Lipopolysaccharide

MTBI	4-Methylthiobutylisothiocyanate
NFκB	Nuclear factor kappa B
NK	Natural killer
NO	Nitric oxide
PBS	Phosphate-buffered saline
PEITC	Phenethyl isothiocyanate
PGE	Prostaglandin E
PMA	Phorbol 12-myristate 13-actate
RT-PCR	Real-time polymerase chain reaction
SCID	Severe combined immunodeficiency
SFN	Sulforaphane
TGF	Transforming growth factor
TNF	Tumor necrosis factor

Introduction

Isothiocyanates (ITCs) are a family of organosulfur compounds (R–N=C=S) that are present in their precursor forms, glucosinolates, in a variety of edible cruciferous vegetables, such as broccoli, cabbage and watercress [1]. Plant injury causes the glucosinolates to be exposed to myrosinase (thioglucosidase), resulting in the formation of ITCs, which are free of the carbohydrate moiety [2]. ITCs can induce apoptosis and cell cycle arrest in transformed cells [1, 3, 4] and prevent angiogenesis in tissue [5, 6]. In addition, ITCs inhibit chemical carcinogenesis by the induction of carcinogen-detoxifying enzymes and inhibition of carcinogen-activating enzymes [1, 3]. Furthermore, the consumption of vegetables containing ITCs has also been associated with a reduced risk of cancer [1, 3].

A few dietary ITCs have been attributed anti-inflammatory abilities [7–9]. They have been shown to reduce carrageenan-induced rat paw edema [8], lessen ear edema formation [10] and induce leukocyte clearance in inflamed mouse skin [11]. In addition, the ITC allyl isothiocyanate suppressed macrophage migration and adipocyte production of tumor necrosis factor (TNF)-α in obesity-induced inflammation [12]. Topical application of the ITC sulforaphane (SFN) alleviated skin blistering in keratin-14-deficient mice by activating the Kelch-like ECH-associated protein 1/nuclear factor-like 2 (Keap1/Nrf2)/antioxidant response element signaling pathway [13] and protected against ultraviolet-induced skin inflammation [14]. Phenylethyl isothiocyanate (PEITC) derivatives and benzyl isothiocyanate (BITC) have been demonstrated to inhibit the production of the proinflammatory factors interleukin (IL)-1β, IL-6, TNF-α, prostaglandin E (PGE)-2, nitric oxide (NO), inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2, that had been induced by phorbol 12-myristate 13-acetate (PMA) or lipopolysaccharide (LPS) in murine RAW264.7 macrophages [10, 15].

Inflammation in the autoimmune skin disease, psoriasis, is regulated by a complex network of interactions between keratinocytes and cells of the immune system [16, 17]. One of the major participants in this skin disease are macrophages, which secrete proinflammatory cytokines, including IL-1β, IL-6, IL-8, IL-23 and TNF-α, as well as the intercellular adhesion molecule-1 (ICAM-1) [17–20]. IL-1 induces phenomena characteristic of psoriasis, such as keratinocyte proliferation, the expression of genes related to aberrant differentiation of keratinocytes [21] and the expression of various endothelial adhesion molecules (including ICAM-1) and chemokines [19], which facilitate the extravasation of immune cells into the dermis and epidermis [18, 19]. TNF-α modulates cell growth and the differentiation of a number of cell types, among them epithelial cells [22]. Moreover, anti-TNF-α treatment has been demonstrated to significantly improve psoriatic lesions in mice and humans and is one of the more effective psoriasis medications known today [17, 18]. Recently, it was mentioned that IL-6, IL-21 or IL-23 (the most effective) in combination with transforming growth factor (TGF)-β1, IL-1β and IL-2 provide the optimum conditions to induce the differentiation of naïve human T cells into TH-17 cells, which are highly associated with autoimmune inflammatory diseases [23]. These data provide evidence that IL-1, IL-6, IL-23, ICAM-1 and TNF-α are important factors in skin inflammatory disease. Substances that influence these factors may have therapeutic properties.

In a former study, a less studied ITC, 4-methylthiobutylisothiocyanate (MTBI, $CH_3S[CH_2]_4NCS$), was found to inhibit the gene expression of the proinflammatory molecules IL-1β, IL-12/23p40, TNF-α and ICAM-1 in human monocytes. It and its oxidized form, SFN (4-methysulfinylbutyl ITC, $CH_3SO[CH_2]_4NCS$), are components of seeds of the cruciferous vegetable rocket (*Eruca sativa*). An extract from these seeds was revealed to contain five times more MTBI than SFN [24]; the latter is an ITC known to have antiproliferative, anticarcinogenic and antiangiogenic activities [3, 24]. In the present investigation, the skin anti-inflammatory potential of MTBI and SFN, alone and/or in combination, was examined in (1) LPS-induced THP-1 monocytes and macrophage-like cells, (2) an inflammation-induced ex-vivo human skin organ culture and (3) a severe combined immunodeficiency (SCID) psoriasiform mouse model.

Materials and methods

Materials

MTBI (purity >95 %), which was synthesized as previously described [24], was generously provided by the

laboratory of Prof. Jacob Vaya, MIGAL-Galilee Technology Center, Israel. Sulforaphane (purity >90 %), was purchased from Sigma-Aldrich (Rehovot, Israel). All other chemicals, unless noted otherwise, were of analytical grade and were obtained from Sigma-Aldrich.

Cell culture

THP-1 human leukemic monocytes were grown in RPMI 1640 medium supplemented with 5 % fetal calf serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin and 100 μg/ml streptomycin (Biological Industries, Beit Haemek, Israel). PMA (5 ng/ml), which is commonly used to cause monocytes to differentiate into macrophages, was added to THP-1 cells for 72 h. The PMA concentration and time period chosen were according to the optimization of Park et al. [25]. Monocytes and macrophage-like cells were activated by incubation with 500 ng/ml LPS for 3 or 4 h, before being harvested for mRNA extraction. MTBI and SFN were diluted in dimethyl sulfoxide (DMSO), prior to their addition to cells. The final concentration of DMSO did not exceed 0.1 % (v/v). All cells were maintained in a standard culture incubator with humidified air containing 5 % CO_2 at 37 °C.

Human skin organ culture

Skin samples were obtained with informed consent from healthy 18–60-year-old women undergoing plastic surgery. They were cut to approximately 0.25 cm^2 and placed in 35-mm diameter Petri dishes, so that the dermal side was in the Dulbecco's modified Eagle medium (DMEM), and the epidermis was exposed to air. LPS (10 μg/ml) was added to the growth medium in order to obtain inflammation. MTBI (5 μl of 0, 0.5 and 1.0 μg/ml in 50 % ethanol) was applied to the epidermis (to achieve 0, 0.0025 and 0.005 μg per skin sample, respectively), after which the skin organ culture was incubated at 37 °C under 5 % CO_2 for 24 h.

Psoriasiform beige SCID mice and experimental design

Psoriasiform beige SCID mouse experiments were conducted at the Ruth and Bruce Rappaport Faculty of Medicine, Technion – Israel Institute of Technology under the supervision of Prof. Amos Gilhar, as previously described [26–29]. Briefly, healthy human skin pieces (thickness 0.4 mm and surface area 1.5 × 1.5 cm) were provided from residual skin of routine plastic surgery procedures from the Plastic Surgery Department of the Rambam Medical Center, with informed consent from patients. The skin pieces were grafted onto thirty beige SCID mice. Four weeks after skin grafting, 1×10^7 allogeneic natural killer (NK)-like-cells, isolated from blood samples of non-treated psoriatic patients with classic plaque psoriasis and cultured in the presence of IL-2 (100 U/ml) for 14 days, were injected into each mouse. Two weeks after the NK-like cell injections, the mice were divided into four groups and treated topically for 14 days as follows: (1) seven mice, negative control, 50 μL vehicle (10 % DMSO in aquacream) twice a day; (2) eight mice, 1 mg MTBI/mouse/day, 50 μL 1.0 % MTBI in vehicle twice a day; (3) eight mice, 1 mg SFN/mouse/day, 50 μL 1.0 % SFN in vehicle twice a day; and (4) seven mice, positive control, i.e., 2 mg dexamethasone/mouse/day, 40 μL 2.0 % dexamethasone in phosphate-buffered saline (PBS), once daily. Two weeks after treatment had begun (4 weeks following the NK-like cell injections), the skin was harvested, and grafts were analyzed by histology.

mRNA expression

mRNA expression was analyzed by real-time polymerase chain reaction (RT-PCR). Total RNA was extracted from cell pellets with TRI Reagent. Each cDNA sample was produced from 1 μg total RNA, which was incubated at 74 °C for 10 min with 100 pmol of oligo dT17 primer. This mixture was added to a 25 μL solution containing 2 mM deoxynucleotides (dNTP) (Larova, Germany), 30 U RNasin, 10 U avian myeloblastosis virus-reverse transcriptase (AMV-RT) and the AMV-RT buffer (Promega, Israel), followed by incubation at 42 °C for 2 h and 52 °C for an additional 1 h. The cDNA of the mRNA transcripts was amplified by a spectrofluorometric thermal cycler (Rotor-Gene™ 6000, Corbett Research, Australia), in a mixture containing specific primers and either Absolute Blue QPCR SYBR Green ROX Mix (ABgene, UK) or KAPA SYBR FAST Universal qPCR Kit (Kapa Biosystems, South Africa), according to the protocol supplied by the manufacturers. For a few of the experiments, the primers for IL-12/23p40, TNF-α and ICAM-1 were purchased (Quantitect Primer Assays, Hilden, Germany). For most of the experiments, the primers employed were: IL-1β (forward) 5′-ggacaggatatggagcaaca-3′, (reverse) 5′-ggcaga ctcaaattccagct-3′; IL-6 (forward) 5′-agagtagtgaggaacaag ccaga-3′, (reverse) 5′-gctacatttgccgaagagccct-3′; IL-12/23p40 (forward) 5′-tcagggccattggactctccgt-3′, (reverse) 5′-c accatttctccaggggcatccg-3′; TNF-α (forward) 5′-gtgatcggcc cccagaggga-3′, (reverse) 5′-cacgccattggccaggaggg-3′; ICAM-1 (forward) 5′-ggaaagcagcaccgccccctt-3′, (reverse) 5′-ctccccgg ggcaggatgact-3′; and the housekeeping gene, β-actin (to which each gene of interest was normalized) (forward) 5′-gcc ctg gac ttc gag caa ga-3′, (reverse) 5′-tgccagggtacatggtggtg-3′. The annealing temperature was 58 °C for the PCR of all genes, except for that of IL-6, which was 65 °C.

Cytokine secretion

IL-1, IL-6, IL-8, TNF-α and IL-12/23p40 secreted into the media were assayed by a highly sensitive solid phase sandwich enzyme-linked immunosorbent assay (mini ELISA Development Kit, PeproTech Inc, Israel) according to the manufacturer's recommendations.

Histology

Histological assessment of the grafts was performed by light microscopy as reported previously [29]. Two blinded observers performed the evaluation, including one who was not aware of the design of the study.

Statistical analysis

Student's t test was employed for statistical analysis. Unless otherwise noted, the two-tailed test was used, and results were considered to be statistically significant if $p < 0.05$.

Results

MTBI combined with SFN had an additive anti-inflammatory effect

MTBI and SFN are present together in rocket seeds. Thus, their combined effect was investigated on monocytes, which are major participants in the inflammatory process. THP-1 monocytes (1.6×10^6 cells/well) were activated with a 2-h LPS treatment, followed by the addition of MTBI and SFN (0.2 or 0.4 μg/ml, each) together or separately. Cells were therefore exposed to LPS for a total of 4 h. In addition, all cells received the same concentration of DMSO. As shown in Fig. 1, MTBI and SFN, alone and in combination, reduced the LPS-induced increase in the proinflammatory cytokine mRNA levels of TNF-α, IL-1β and IL-12/23p40. This decrease in the cytokine transcription level was relative to the ITC concentration added to the cells, although the difference was not always significant. Nevertheless, in all cases, the effect between SFN and MTBI tended to be additive, as demonstrated by the similarity between the cytokine mRNA levels attained from the combined SFN-MTBI (0.2 μg/ml each, together equaling 0.4 μg/ml) and those obtained for either MTBI (0.4 μg/ml) or SFN (0.4 μg/ml) separately.

No significant difference in the anti-inflammatory activity of MTBI and SFN was observed, except in the case of IL-1β (Fig. 1a). At the dose of 0.2 μg/ml, SFN caused a greater reduction in the LPS-induced IL-1β mRNA level than did MTBI ($p < 0.03$).

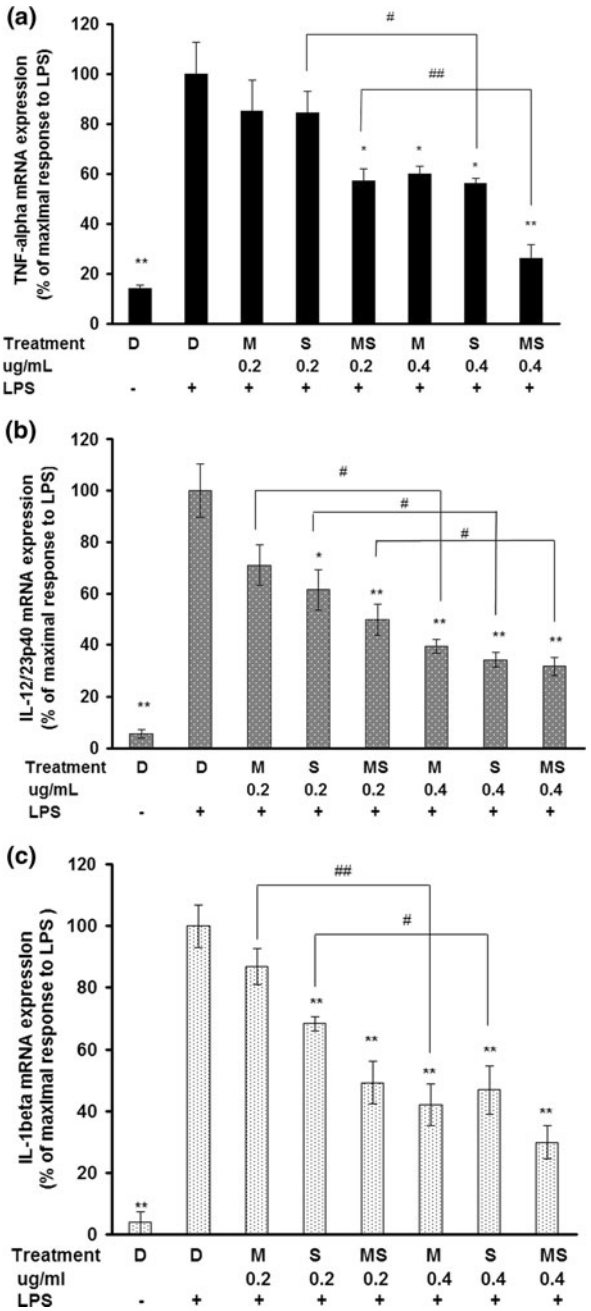

Fig. 1 The combined MTBI-SFN treatment had an additive anti-inflammatory effect. Inflammation was induced in THP-1 monocytes (1.6×10^6 cells/well) by LPS (500 ng/ml). Two hours later, MTBI (*M*, 0.2 or 0.4 μg/ml), SFN (*S*, 0.2 or 0.4 μg/ml), MTBI and SFN (*MS*, 0.2 or 0.4 μg/ml each) or DMSO (*D*, 0.008 %) was added to the cells, which were incubated for an additional 2 h (with a total of 4 h LPS). Transcription of proinflammatory cytokines (**a** TNF-α, **b** IL-12/23p40, **c** IL-1β) was determined by real-time PCR, and cytokine mRNA levels were normalized with those of β-actin. Results are expressed as mean ± SE, $n = 5$–6. $^{*}p < 0.05$, $^{**}p < 0.005$: significant difference from the LPS controls; $^{\#}p < 0.05$, $^{\#\#}p < 0.005$: significant difference between treatments of different concentrations. There was a significant difference between the combined treatment and each single MTBI or SFN treatment in the case of TNF-α ($p < 0.05$ for 0.2 μg/ml and $p < 0.005$ for 0.4 μg/ml) and IL-1β ($p < 0.05$ for 0.2 μg/ml)

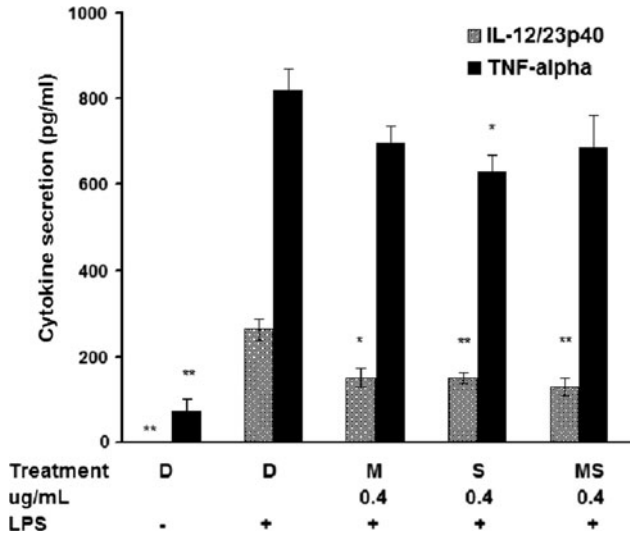

Fig. 2 The MTBI–SFN combination lowered IL-12/23p40 secretion significantly and TNF-α secretion only slightly in monocytes. Inflammation was induced in THP-1 monocytes (1.6×10^6 cells/well) by LPS (500 ng/ml). Two hours later, MTBI (*M*, 0.4 μg/ml), SFN (*S*, 0.4 μg/ml), MTBI and SFN (*MS*, 0.4 μg/ml each) or DMSO (*D*, 0.008 %) were added to the cells, which were incubated for an additional 2 h (with a total of 4 h LPS). Secretion of proinflammatory cytokines from the cells to the medium was determined by sandwich ELISA. Results are expressed as mean ± SE, $n = 6$. Significant difference from the LPS controls: $*p < 0.05$, $**p < 0.005$

The effect of the combination of MTBI and SFN was also tested on the cellular secretion of TNF-α and IL-12/23p40. Both MTBI and SFN, together and separately, brought about a decrease in the LPS-induced IL-12/23p40 secretion, but had only a minimum effect on the induced TNF-α secretion (Fig. 2). The effect of the ITCs together was the same as each separately (Fig. 2).

MTBI and SFN downregulated transcription of proinflammatory psoriasis-related factors in LPS-induced macrophage-like cells

Among the cells residing in and migrating to the skin during skin inflammation are macrophages, which have a key role in psoriasis [17]. They were thus chosen to probe the effect of ITCs on inflamed skin. Human macrophage-like THP-1 cells were activated by LPS (500 ng/ml) for 1 h to induce an inflammatory reaction. Subsequently, MTBI (0.8 μg/ml = 5 μM: approximate IC_{50} of MTBI), SFN (0.8 μg/ml: 4.5 μM) or 0.01 % DMSO (control) were added, and cells were incubated for an additional 2 h, so that all treatment groups were exposed to LPS for a total of 3 h (and the same concentration of DMSO). LPS induced an increase in ICAM-1, IL-1β, IL-6, IL-12/23p40 (the subunit common to IL-12 and IL-23) and TNF-α transcription, as demonstrated by the DMSO-LPS controls (not shown). The LPS-induced increase in IL-6, IL-12/23p40 and TNF-α mRNA expression in the macrophage-like cells was significantly downregulated by MTBI and SFN (Table 1). MTBI also caused a decrease in the LPS-induced transcription of IL-1β (as opposed to SFN) (Table 1). However, MTBI and SFN had no effect on ICAM-1 transcription in these LPS-induced cells (Table 1). It is notable that in macrophages which had not been activated with LPS, 2-h treatments of both ITCs had no significant effect on IL-6, IL-12/23p40, TNF-α or ICAM-1 transcription, although there was a reduction in IL-1β transcription (Table 2). Four-hour ITC treatments in non-induced macrophage-like cells did not have a significant effect on TNF-α and IL-12/23p40, but mRNA expression of ICAM-1 was increased by both, and that of IL-1β and IL-6 was decreased, by MTBI and SFN, respectively (Table 2).

Table 1 Treatment with MTBI and SFN (0.8 μg/ml) downregulates transcription of proinflammatory molecules in LPS-induced macrophage-like cells (PMA-treated monocytes)

	mRNA expression (% of maximal response to LPS)[a]			
	LPS before ITC		LPS after ITC	
	MTBI ($n = 5$–6)	SFN ($n = 6$)	MTBI ($n = 9$–12)	SFN ($n = 8$–12)
IL-12/23p40/β-actin	75 ± 9*	60 ± 6**	60 ± 9**	42 ± 7**
TNF-α/β-actin	64 ± 3**	63 ± 3**	85 ± 4*	79 ± 8*
ICAM-1/β-actin	84 ± 6	86 ± 8	105 ± 6	96 ± 8
IL-1β/β-actin	78 ± 3**	85 ± 6	113 ± 9	78 ± 6*
IL-6/β-actin	75 ± 6*	75 ± 8*	57 ± 5**	29 ± 2**

The increases in percent between the DMSO negative controls and DMSO-LPS positive controls were significant ($p < 0.001$): IL-12/23p40: 1100; TNF-α: 1060; ICAM-1: 420; IL-1β–220; IL-6: 1660

[a] Mean ± SE, $*p < 0.05$, $**p < 0.005$ difference from maximal response to LPS

Table 2 Treatment with MTBI and SFN (0.8 μg/ml) has a minimal effect on transcription of proinflammatory molecules in non-induced macrophage-like cells

	mRNA expression (% of DMSO control)[a]			
	2 h		4 h	
	MTBI ($n = 6$)	SFN ($n = 6$)	MTBI ($n = 9$–12)	SFN ($n = 9$–12)
IL-12/23p40/β-actin	76 ± 12	98 ± 20	81 ± 16	74 ± 10
TNF-α/β-actin	90 ± 7	95 ± 15	103 ± 5	110 ± 9
ICAM-1/β-actin	95 ± 11	97 ± 10	129 ± 7*	143 ± 8**
IL-1β/β-actin	77 ± 3**	86 ± 3*	61 ± 12*	89 ± 19
IL-6/β-actin	108 ± 11	126 ± 24	84 ± 7	69 ± 5**

[a] Mean ± SE, $*p < 0.05$, $**p < 0.005$ difference from DMSO control

In order to learn if MTBI could also prevent the induction of inflammation (as opposed to interfering with the ongoing inflammation process, above), an experiment was conducted on macrophage-like THP-1 cells, as above, except that this time MTBI and SFN were added to the cells for 1 h before the subsequent 3-h treatment of LPS (the same LPS exposure as in the above experiment). Both MTBI and SFN prevented the inflammatory response induced by LPS, as demonstrated by the significantly low mRNA levels of IL-6, IL-12/23p40 and TNF-α in cells (Table 1). However, pretreatment with MTBI did not prevent the induction of ICAM-1 (similarly to SFN) or that of IL-1β (in contrast to SFN). These data imply that these ITCs are relatively specific to cells involved in the inflammatory process, and that MTBI and SFN are capable of hindering inflammation.

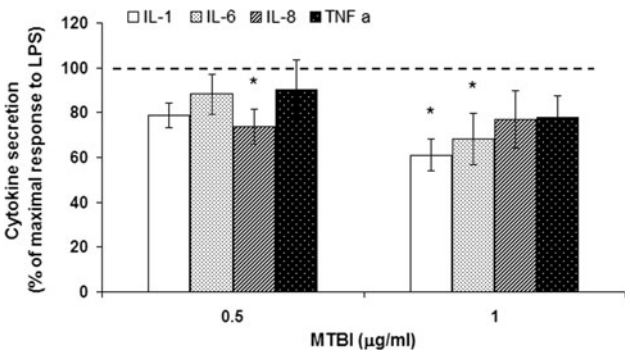

Fig. 3 MTBI caused a decrease in LPS-induced proinflammatory cytokine secretion in an ex-vivo skin organ culture. Skin samples were treated in the growth media with LPS (10 μg/ml) and topically with 5 μl MTBI (0, 0.5, 1.0 μg/ml in 50 % ethanol, as a carrier). Twenty-four hours later, cytokines secreted into the medium were analyzed by ELISA. Results are expressed as mean ± SE, $n = 13$–20. $*p < 0.05$: significant difference from the control

MTBI brought about a decrease in the proinflammatory cytokine secretion in inflamed human skin organ culture

An assessment of the overall effect of MTBI on skin inflammation was carried out on an ex-vivo LPS-induced human skin organ culture (epidermis and dermis). When this inflammation-induced skin organ culture was treated topically with MTBI (1 μg/ml), there was a significant decrease in the induced secretion of the proinflammatory cytokines IL-1 and IL-6 (Fig. 3). A decrease in IL-8 was significant at the lower dose (0.5 μg/ml MTBI), and the LPS-induced level of TNF-α tended to decrease after MTBI treatment (72.3 % of the control, $p = 0.057$). The ITCs had no significant effect on the level of IL-1, IL-6, IL-8 and TNF-α in the normal non-inflamed skin (not shown).

MTBI and SFN demonstrated a beneficial effect in a psoriasiform SCID mouse model

The psoriasiform skin SCID mouse model is one of the accepted models for testing psoriatic drugs. The model is based on beige SCID mice that are xenografted with normal human skin and injected with psoriatic human NK-like cells. The resulting psoriasiform skin is characterized by acanthosis (thickening of the stratum spinosum), enhanced epidermal proliferation, hyperkeratosis, parakeratosis (lack of nuclei in the stratum corneum), dermal lymphocytic infiltrate and some areas with, and others without, the granular layer. Elongation of rete ridges is also observed in most cases and vascular dilatation associated with a perivascular lymphocytic infiltrate is noted in the papillary dermis [26, 28]. Although most histological features are similar to psoriasis, there are also some signs of dermatitis, which exclude defining the condition as pure psoriasis [27]. These above psoriasiform features were found in grafts of all mice from the vehicle (10 % DMSO in aquacreme)-treated group; i.e., none of this group showed recovery from this condition (0/7) (Table 3). However, grafts from mice treated with SFN (3/8) and MTBI (2/8) demonstrated a complete or partial recovery from this psoriasiform phenotype (Table 3). As expected, normal skin grafts from mice treated with dexamethasone (2 mg/mouse/day) showed a complete recovery (7/7).

Table 3 Histological evaluation of MTBI- and SFN-treated psoriasiform human skin xenografts in beige SCID mice

Histological features		MTBI (1 mg/kg/day)	SFN (1 mg/kg/day)	Vehicle
Recovery	Complete	2/8	2/8	0/7
	Partial	0/8	1/8	
Psoriasiform		6/8	5/8	7/7

Discussion

This study further corroborates our hypothesis that ITCs may be beneficial in the treatment of skin inflammatory diseases. The two ITCs, MTBI and SFN, reduced the expression and secretion of proinflammatory cytokines in human monocytes, macrophage-like cells and inflamed skin. In addition, they brought about recovery from the psoriasiform (psoriasis-like) skin phenotype in a known human skin psoriasis model.

MTBI and SFN significantly downregulated the LPS-induced increase in IL-6, IL-12/23p40 and TNF-α mRNA expression in macrophage-like cells, corresponding to results obtained in THP-1 monocytes in our former investigation [24]. MTBI also caused a decrease in the LPS-induced transcription of IL-1β (as opposed to SFN). Others have reported that SFN pretreatment also reduced IL-1β and TNF-α transcription in mouse peritoneal macrophages [30]. In contrast to the MTBI-generated downregulation of ICAM-1 in LPS-treated monocytes found in a previous study [24], MTBI and SFN had no effect on ICAM-1 transcription in LPS-induced macrophage-like cells.

When MTBI and SFN were added as a combined treatment to LPS-activated THP-1 monocytes, the induced proinflammatory cytokine levels of IL-1β, IL-12/23p40 and TNF-α were downregulated, and the levels of IL-12/23p40 and TNF-α secreted from the cells into the medium was decreased. Their combined effect on transcription, but not on the secretion level, tended to be both additive and relative to the ITC concentration added to the cells. Possibly, both ITCs act by means of the same mechanism. They have similar aliphatic side chains that could support a similar reaction. Interestingly, both are metabolically interconverted in humans in vivo (oxidation–reduction of the sulfur atom), implying similar biological activity [31]. In an investigation of the anti-inflammatory mechanisms of PEITC derivatives (some more active than PEITC itself), the chemical structure of the derivative had an effect on the activity, which was found to be elicited by means of NFκB transactivation suppression [15]. Nevertheless, Prawan et al. [15] suggested that the receptor site that interacts with ITCs may be relatively nonspecific.

Explants of human skin, which can be grown in medium, are often termed ex-vivo skin organ culture. This type of culture has been employed as a basis for skin inflammation studies [32], and recently in a psoriasis model [33]. It provides indications regarding the effect of drugs or substances on the whole organ, in this case skin, without necessitating the killing of animals. In the present study, LPS was used to induce inflammation in such an ex-vivo skin organ culture. This induced inflammation was significantly lessened by MTBI, as shown by the decrease in the secretion of inflammation-related cytokines IL-1, IL-6 and IL-8.

Psoriasis, which is possibly the most prevalent immune-mediated skin disease in adults, is characterized by red scaly raised plaques resulting from aberrant differentiation and hyperproliferation of keratinocytes, as well as angiogenesis [17, 18]. Histological features, which distinguish psoriatic skin from normal skin, include very elongated epidermal rete, a reduced or nonexistent granular layer, acanthosis and the retention of nuclei in the stratum corneum [18]. Psoriasiform skin differs slightly from psoriatic skin in that it has irregular acanthosis, a thick granular layer and epidermal spongiosis [27]. Nevertheless, the psoriasiform SCID mouse model is an accepted preclinical model for assessing the potential of psoriatic drugs [26, 28]. This psoriasiform SCID mouse model employs SCID mice that are transplanted with normal human skin, and subsequently injected with IL-2-stimulated human psoriatic NK-like cells [26–28]. It is derived from a psoriasis mouse model, which uses psoriatic human skin [26, 28]. MTBI and SFN brought about a partial or complete recovery from induced psoriasiform skin in 2/8 and 3/8 mice, respectively, as compared to 0/7 in the vehicle-treated mice. Others that have used this model have shown that cyclosporine A (a drug widely used in psoriasis) caused 2/7 and 1/7 mice to completely and partially recover, respectively [26], suggesting that ITCs are as effective as cyclosporine. Although these experiments were carried out on a relatively small number of mice, they indicate that MTBI and/or SFN may be valuable in the therapeutics of psoriasis.

The proinflammatory factors examined in this study are key participants in the skin inflammatory process and are upregulated in psoriatic lesions [16]. TNF-α neutralizing agents are effective in the treatment of human psoriasis and alleviate psoriasiform skin inflammation in mice [17]. An antibody of IL-12/23p40 has been demonstrated to have clinical efficacy in the treatment of psoriasis [34]. Moreover, the genes encoding for IL-12/23p40 and the IL-23 receptor have been reported to be psoriasis-risk genes [34–36]. Thus, these data, which reveal MTBI to have a suppressive effect on major proinflammatory cytokines, imply that MTBI may be capable of influencing the inflammation process in skin diseases.

Conclusion

In this study, which was carried out in vitro on monocytes and macrophage-like cells, ex vivo in inflamed human skin and in vivo in the psoriasis SCID mouse model, rocket seed ITCs reduced inflammatory activity and lessened psoriasiform formation. Results from all three models indicate the potential of ITCs as biological agents in the therapy of psoriasis and inflammation-related skin diseases.

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Conflict of interests The authors declare that they have no conflicting interests.

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