


Can topical cefazolin be an useful treatment for psoriasis?

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ABSTRACT

Introduction: A better understanding of psoriasis pathogenesis resulted in significant development of new, effective anti-psoriatic therapies, albeit new treatment options, especially topical ones, are still awaited. In vitro studies have shown that cefazolin, the first-generation cephalosporin, has the properties of a specific inhibitor of several pro-inflammatory cytokines.

This study aimed to evaluate the effects of cefazolin in 3D human psoriasis skin model and to assess the efficacy and tolerability of topical cefazolin in comparison to topical hydrocortisone butyrate in the treatment of psoriasis.

Material and methods: Commercially available 3D human psoriasis skin model was used to evaluate the effect of cefazolin on psoriasis-related gene expression: human β -defensin 2 (HBD2, DEFB-4A), psoriasin (S100A7) and skin-derived peptidase inhibitor 3 (PI3), as well IL-6 and IL-8 secretion. H&E staining was performed to evaluate tissue morphology. The clinical test was an open-label, comparative, left to right study with an active comparator. Ten adult subjects with psoriasis were asked to apply 5% cefazolin emulsion twice daily on the psoriatic plaques located in extensor area of the right elbow, and 0.1% hydrocortisone butyrate ointment on the same body area on the opposite side. The treatment continued for 7 days. The disease severity was assessed according to the modified PASI (mPASI) and Investigator Global Assessment (IGA). Patients were also asked to rate concomitant subjective symptoms, treatment tolerability and global therapeutic effect.

Results: Cefazolin down-regulated gene expression of HBD2 (DEFB-4A) and psoriasin (S100A7) and did not affect PI3. In the tested conditions the drug did not reduce IL-6 and IL-8 secretion. Histological evaluation revealed a slight thinning of the epithelium in tissues treated with cefazolin. In patients, both of the treatments resulted in a significant reduction of psoriatic plaques, although the therapeutic effect was significantly better for hydrocortisone butyrate ointment. Both drugs equally significantly reduced pruritus intensity. The treatment with cefazolin was well tolerated and any significant adverse events were observed.

Conclusions: Cefazolin can be considered an interesting therapeutic option as a topical immunomodulatory, anti-inflammatory drug, but its anti-psoriatic properties have to be confirmed in well-designed, prospective and vehicle-controlled studies.

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INTRODUCTION

Psoriasis is a chronic, inflammatory skin disease affecting about 1–3% of the general population. The pathogenesis of psoriasis is still not fully elucidated, but it seems that immunological, genetic and some environmental factors play the major role [1]. A better understanding of psoriasis pathogenesis resulted in huge progress in the development of new, effective anti-psoriatic therapies. Besides many well-known drugs such as methotrexate, cyclosporin A or acitretin, providing treatment of satisfactory effectiveness, but burdened with numerous side effects, there are biological drugs with higher selectivity, better clinical effectiveness

and a superior safety profile. These include TNF- α , IL-17, and IL-23 inhibitors. Due to the advancements in therapeutic options, currently, the therapeutic goal in psoriasis is to achieve complete clearance of skin lesions in the shortest possible time and to maintain this condition as long as possible [2].

However, it is worth remembering that approximately 80% of patients could sufficiently benefit from topical therapy. Yet, the progress in the field of topical treatment has not been spectacular, and many old topical drugs are characterized by a number of insufficiencies. Difficulties in the daily use of anthralin ointment, the growing tendency

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among patients to demonstrate steroid phobia, and also the addiction and abuse of topical corticosteroids with a whole range of related side effects incline to the search for new molecules or the use of already known compounds that could potentially show the desired anti-inflammatory, anti-proliferative or keratolytic effect and broaden the spectrum of available topical therapy options.

Recent *in vitro* studies have demonstrated a potential potent anti-inflammatory effect of cefazolin [3]. It could be speculated that cefazolin may act as an inhibitor for cytokines dependent on γ c receptor, *i.e.* IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 [3]. *In silico*, molecular docking unveiled two potential cefazolin binding sites within the IL-2/IL-15R β subunit and two within the γ c subunit. *In vitro*, cefazolin decreased proliferation of PBMC (peripheral blood mononuclear cells) following IL-2, IL-4 and IL-15 stimulation, reduced production of interferon (IFN)- γ , IL-17 and TNF- α in IL-2- and IL-15-treated PBMC and in IL-15 stimulated natural killer (NK) cells, attenuated IL-4-dependent expression of CD11c in monocyte-derived dendritic cells and suppressed phosphorylation of JAK3 in response to IL-2 and IL-15 in PBMC, to IL-4 in TF-1 (erythroleukemic cell line) and IL-21 in NK-92 (NK cell line) [3, 4]. These observations prompted the interest to test, whether the *in vitro* anti-inflammatory properties of cefazolin could also be confirmed *in vivo*. In the current study, the potential anti-psoriatic effect of cefazolin emulsion applied topically was evaluated.

MATERIAL AND METHODS

In vitro experiments and their analyses were performed at MatTek Corporation (MatTek Corporation, Ashland, MA, USA).

The 3D psoriasis tissue model

3D human psoriatic tissue model (SOR-300-FT) developed by MatTek Corporation (MatTek Corporation, Ashland, MA, USA) was used to evaluate the effect of cefazolin (sodium salt, Sigma Aldrich). The tissues were cultured on microporous membrane cell culture inserts and grown at the air-liquid interface under standard culture conditions (37°C, 5% CO₂). Cefazolin or appropriate controls were dosed basolaterally. Before each application (at time 0 and 48 h), tissues were washed 3 times by rinsing in Dulbecco's phosphate-buffered saline (DPBS).

Quantitative PCR

Quantitative PCR (qPCR) analysis was performed on tissues harvested following 96 h incubation with cefazolin or controls (2-time repeat application). RNA was isolated from the tissues following MatTek's standardized RNA isolation protocol. The concentration, integrity, and purity of

RNA were assessed using the Experion System (Bio-Rad). cDNA was generated using the Qiagen RT2 First Strand Kit (cat#330404). Relative gene expression was measured using Qiagen RT2 SYBR Green qPCR Mastermix and Qiagen RT2 primers. The analysis was carried out using Bio-Rad CFX software.

Cytokine release measurement

Cytokine release was measured in tissue supernatants harvested after 48 h (single application) and after 96 h (2-time repeat application) culture with cefazolin. Cytokine analysis was performed using R&D Systems Human IL-6 Quantikine ELISA (cat# D6050) and R&D Systems Human IL-8 Quantikine ELISA (cat# D8000C) using a BioTek plate reader at 450 nm.

Histology

3D psoriasis tissue models were exposed to cefazolin for 8 days (2-time repeat application of 96 h), then fixed with formalin, embedded in paraffin and stained with hematoxylin and eosin (H&E) using standard protocols.

Patients

The study involved 10 adult patients (4 women and 6 men) hospitalized for plaque-type psoriasis in the Department of Dermatology in Rzeszów, Poland. The detailed characteristics of the studied subjects are presented in Table 1.

Tested product

The tested product was an emulsion containing 5% cefazolin. Other components were as follows: white petrolatum, water, cetyl stearyl alcohol, Brij™ S721, urea, phosphate buffer saline, phenoxyethanol, and propylene glycol.

Table 1. Patients' characteristics

Females	4
Males	6
Age	52.7 ± 10.7 (37–72)
Duration of psoriasis (years)	19.6 ± 13.3 (0.5–40)
Duration of the current psoriasis exacerbation (weeks)	83.8 ± 149.0 (2–469)
Family history of psoriasis:	
Yes	1
No	9
Prior treatment of psoriasis	
– Topical therapy	10
– UVB 311 nm	5
– PUVA	1
– Acitretin	2
– Methotrexate	2
– Cyclosporin A	3
– Fumaric acid esters	1

Table 2. Psoriasis-related gene expression of the SOR-300-FT tissues following 96 h exposure to cefazolin

Treatment		HBD2	Psoriasis	PI3
DPBS (negative control)		1.0	1.0	1.0
Water (vehicle control)		1.2	-1.3	-1.7
Calcipotriol (2.5 µg/mL) (positive control)		-15.2	-3.8	-3.8
Cefazolin (µM)	1000	-3.9	-1.4	-1.0
	600	-1.5	1.1	1.0
	400	-1.2	1.0	1.1

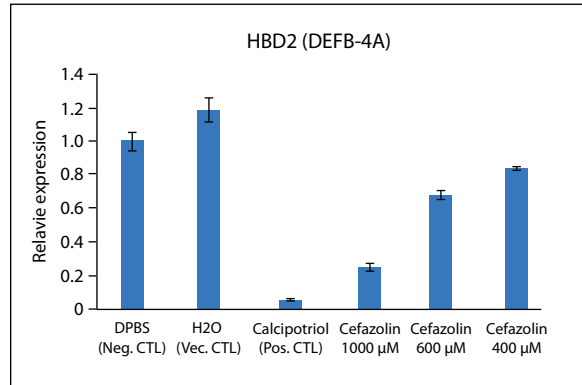
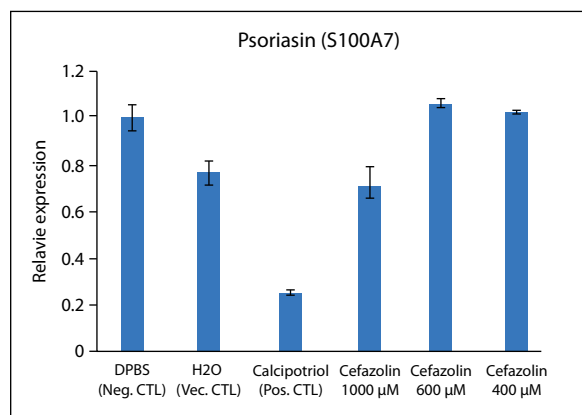
DPBS — Dulbecco's phosphate-buffered saline; HBD2 — human β -defensin 2; PI3 — skin-derived peptidase inhibitor 3

Study design

This was an open-label, comparative left/right study with an active comparator. After providing informed consent, all subjects were asked to apply 5% cefazolin emulsion twice daily on the psoriatic plaques located in extensor area of the right elbow, and 0.1% hydrocortisone butyrate ointment (the active comparator — a medium potent corticosteroid) (Laticort® ointment, Bausch Health, Rzeszów, Poland) in the same area on the opposite side. The treatment was given for 7 days. The disease severity was assessed according to the modified Psoriasis Area and Severity Index (sum of the intensity of erythema, induration and desquamation – mPASI) and the Investigator Global Assessment (IGA) before the treatment (Day 0) and at Day 3, Day 5 and Day 7 [5]. Also, patients were asked to rate the intensity of subjective symptoms (pruritus, burning, stinging, and pain) within the treated areas according to the Numerical Rating Scale (NRS) [6]. The tolerability of the treatment was assessed by both, the patient and the investigator, at Day 3, Day 5 and Day 7 with the three-step scale (good, moderate, poor). At the end of the study, patients were asked about the outcome of the treatment with the 5% cefazolin emulsion using following descriptors: worsening, no improvement, slight improvement, marked improvement, and clearance of the skin lesions. Photographic pictures of observed skin lesions were taken on Day 0, Day 3, Day 5 and Day 7.

Statistical analysis

All data were analyzed statistically with Statistica 12.0 (Statsoft, Krakow, Poland). Means, standard deviations (SD), median values and frequencies were calculated. The differences between achieved results were analyzed using Fisher's exact test, Wilcoxon's signed-rank test or Friedman's analysis of variance (ANOVA). The results were considered statistically significant if p-value was less than 0.05.

**Figure 1.** qPCR results showing the effect of cefazolin on human β -defensin 2 (HBD2) gene expression levels in the 3D psoriatic tissue model following 96 h treatment (\pm SEM, N = 2); DPBS — Dulbecco's phosphate-buffered saline; CTL — control**Figure 2.** qPCR results showing the effect of cefazolin on psoriasis gene expression levels in the 3D psoriatic tissue model following 96 h treatment (\pm SEM, N = 2); DPBS — Dulbecco's phosphate-buffered saline; CTL — control

RESULTS

The effects of cefazolin in a 3D psoriasis tissue model

The effects of 400 μ M, 600 μ M and 1000 μ M cefazolin on human β -defensin 2 (HBD2), also known as Defensin Beta 4A (DEFB-4A), psoriasis, also known as S100 Calcium Binding Protein A7 (S100A7) and skin-derived peptidase inhibitor 3 (PI3) gene expression are presented in Table 2 and Figure 1 and 2. Water served as vehicle control, DPBS as a negative control and calcipotriol (2.5 μ g/mL) as a positive control. At 1000 μ M, the highest tested concentration, cefazolin induced a significant reduction in HBD2 expression (3.9 fold), a slight reduction in psoriasis expression (1.4 fold) and no significant reduction in PI3 gene expression. At 600 μ M, cefazolin slightly reduced HBD2 expression (1.5 fold) and did not affect psoriasis and PI3 expression.

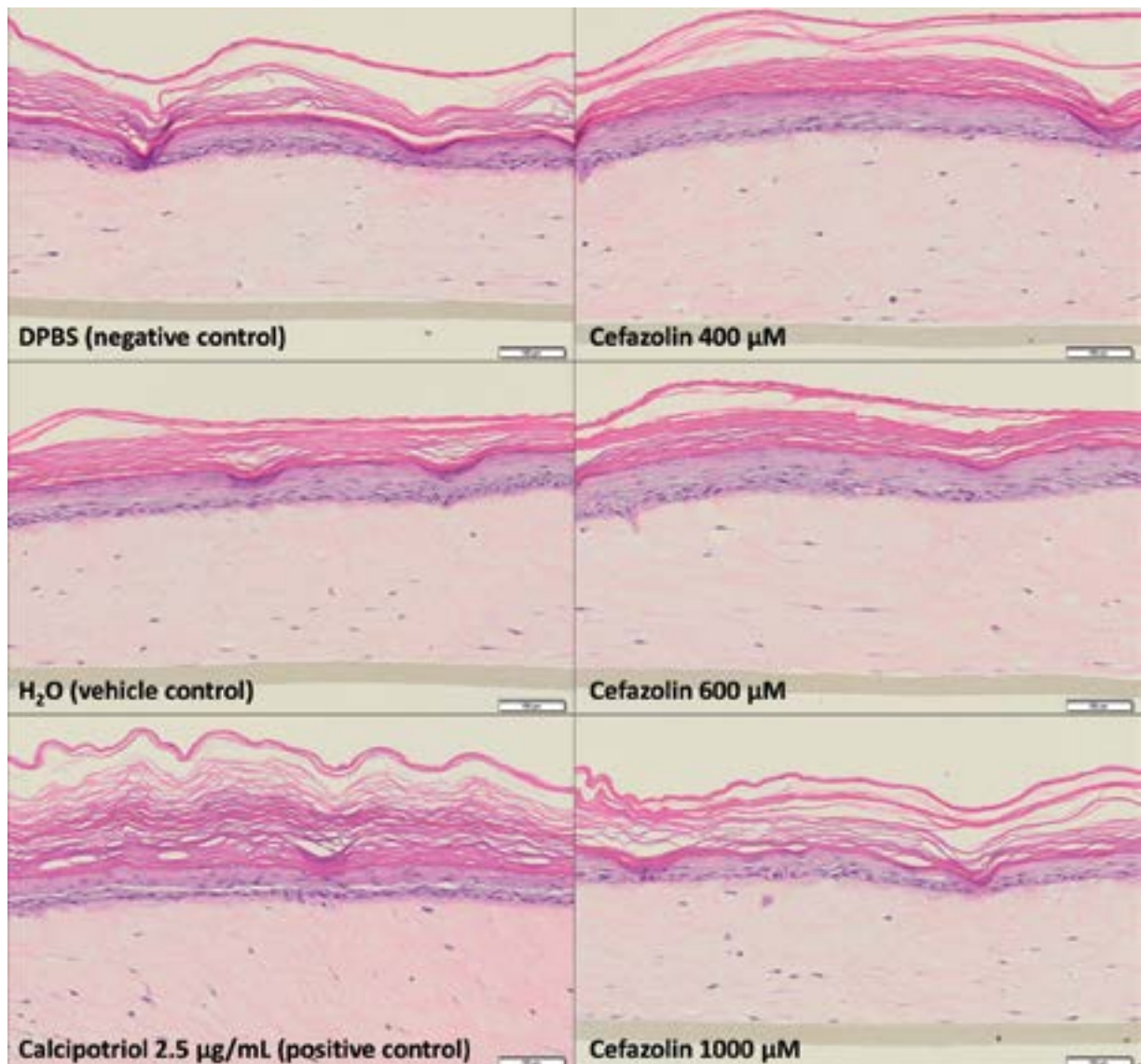


Figure 3. Histological evaluation of hematoxylin and eosin-stained cross-sections revealing slight thinning of the epidermis in 1000 µM cefazolin treated samples; DPBS — Dulbecco's phosphate-buffered saline

qPCR analysis showed no significant reduction in psoriasis-related gene expression for 400 µM cefazolin.

Cefazolin showed no effect on IL-6 and IL-8 release after for 48 h and for 96 h treatment (data not shown).

Histological evaluation of H&E stained cross-sections of the treated tissues revealed slight thinning of the epithelium in 1000 µM cefazolin samples (Fig. 3).

Disease severity

Both treatment arms led to a significant reduction of psoriatic plaques (Fig. 4). The details of the change of disease severity are shown on Figures 5 and 6 (mPASI for cefazolin: $p = 0.002$, mPASI for hydrocortisone butyrate: $p < 0.001$,

Figure 5; IGA for cefazolin: $p = 0.01$, IGA for hydrocortisone butyrate: $p < 0.001$, Fig. 6). However, 0.1% hydrocortisone butyrate ointment provided a significantly better reduction of psoriatic lesions at each study visit compared to 5% cefazolin emulsion ($p < 0.05$, Fig. 5 and 6).

Subjective sensations

Most patients suffered from mild pruritus in the tested areas (mean NRS: 1.8 ± 1.3 points). Both treatments led to a significant reduction of pruritus intensity ($p = 0.001$ for both treatment arms) with no significant difference between right and left side (p values ranged from 0.42 to 1.0) (Fig. 7). As other sensations (pain, burning, and stinging)



Figure 4. Comparison of the psoriatic plaque reduction during 0.1% hydrocortisone butyrate and 5% cefazolin treatment

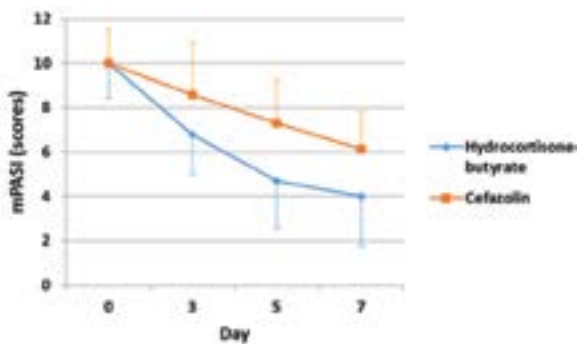


Figure 5. Comparison of the modified Psoriasis Area and Severity Index (mPASI) reduction during the treatment with 5% cefazolin ointment and 0.1% hydrocortisone butyrate ointment

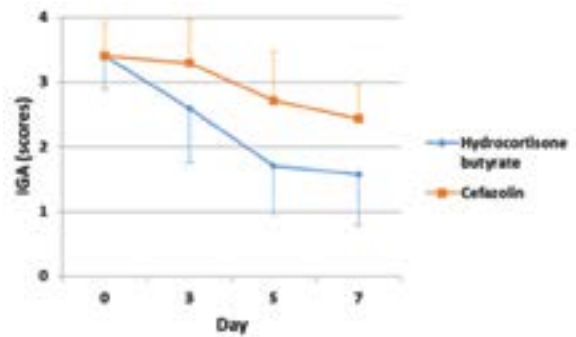


Figure 6. Comparison of the Investigator Global Assessment (IGA) reduction during the treatment with 5% cefazolin ointment and 0.1% hydrocortisone butyrate ointment

were only reported by single patients and were of minimal intensity (NRS = 1–2 points), analysis on them has not been performed.

Tolerability and final treatment outcome

Both treatments arms were well tolerated. No serious adverse events were observed during the 7-day treatment. Only 1 patient reported slight burning in the area of 5% cefazolin emulsion application at day 3 (Fig. 8a and 8b).

Eight out of ten patients reported the final treatment outcome. Three patients assessed the final result of the 7-day treatment with 5% cefazolin as marked improvement, 4 as slight improvement and 1 as no improvement.

DISCUSSION

Recent years provided development of new systemic therapies for psoriasis, however, with the rather slow progress of topical antipsoriatic therapies [7]. The need for

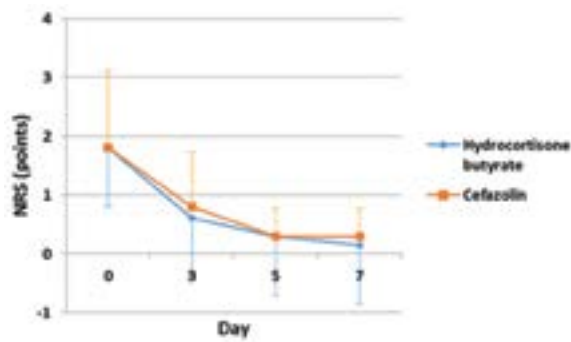


Figure 7. Comparison of the improvement of pruritus intensity during the treatment with 5% cefazolin ointment and 0.1% hydrocortisone butyrate ointment; NRS — Numerical Rating Scale

a broader spectrum of therapeutic options fuels the search for new molecules with local anti-inflammatory, antiproliferative or keratolytic properties. Recent *in vitro* studies have shown that cefazolin, an antibiotic belonging to the first generation of cephalosporins, could be considered as a potential inhibitor of all γ c family cytokines, *i.e.* IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 [3]. Novel insights into cefazolin action may expedite repositioning of the drug to the area of immunotherapeutic intervention.

Results presented in this study suggest that cefazolin holds promise for psoriasis patients. As shown using the 3D psoriasis tissue model, cefazolin reduces the expression of two genes most highly expressed in psoriasis lesion skin: antimicrobial protein HBD2 (DEFB-4A) which is among the top 10 and psoriasin (S100A7) — one of the top 100 of the upregulated genes [8]. HBD2 is a downstream marker of IL-17A signalling and psoriasis disease severity. It has also been linked to keratinocyte hyperproliferation and chemoattraction of neutrophils — both important mechanisms in the pathogenesis of psoriasis [9]. Psoriasin (S100A7) amplifies the inflammatory process in psoriatic skin, perpetuating the disease phenotype [10]. Both HBD2 and psoriasin are considered as a therapeutic target in psoriasis. Further studies are necessary to fully elucidate the mechanism of the observed alleviation of psoriatic phenotype.

The current, preliminary study compared the effectiveness of topically applied cefazolin with hydrocortisone butyrate — a corticosteroid with the moderate anti-inflammatory potential. It was shown that both treatment options resulted in a reduction of psoriatic plaques, although the therapeutic effect was more clearly seen with hydrocortisone butyrate ointment. It should be emphasized, however, that both drugs were used for a relatively short time (only 7 days), the study was not blinded, and the assessed patient population included only 10 people. Although the results seem promising and cefazolin may

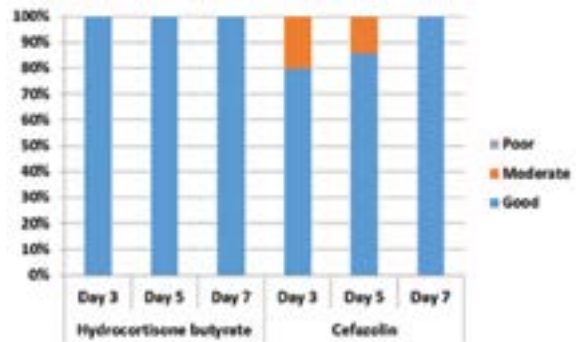


Figure 8a. Treatment tolerability assessed by patients

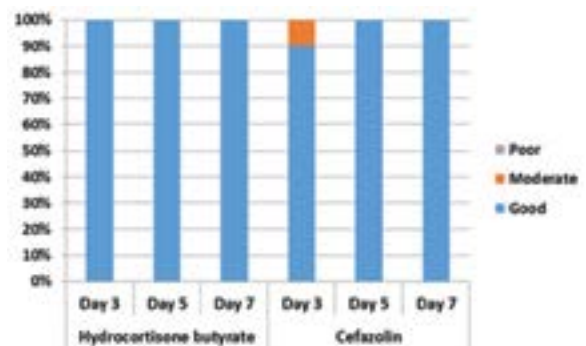


Figure 8b. Treatment tolerability assessed by the physician (MKK)

become an interesting alternative to current topical preparations, the authors' treatment results should be evaluated with caution. The observed anti-psoriatic effect after the use of cefazolin may be the result of a specific drug effect, however, it cannot be excluded that the observed effect resulted from the skin moisturizing caused by the vehicle. To reliably confirm the anti-psoriatic properties of cefazolin clinically, placebo-controlled, preferably double-blind, studies should be performed with a significantly larger number of participants, and with a significantly longer observation period.

Summarizing, cefazolin seems to be an interesting therapeutic option as a topical anti-inflammatory drug, but its anti-psoriatic properties have to be confirmed in well-designed, prospective, and vehicle-controlled studies.

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