Clinical Pharmacology
in Drug Development
2020, 9(5) 582–592

DOI: 10.1002/cpdd.736

Comparison of In Vitro and In Vivo Percutaneous Absorption Across Human Skin Using BAY1003803 Formulated as Ointment and Cream

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Abstract

Direct comparisons between skin absorption data and clinical pharmacokinetic data are rare. Here we use the lipophilic nonsteroidal selective glucocorticoid receptor agonist BAY1003803 to make such a comparison. The objective is to find the extent to which measurements of skin permeation in vitro can be used to predict the corresponding permeation in vivo for human pharmacokinetics of topically applied substances. BAY1003803 was prepared in various formulations: ointment, hydrophilic cream, lipophilic cream, and milk. Its ability to permeate healthy human skin was measured in vitro in static diffusion cells, and percutaneous absorption as well as dermal delivery was measured thereafter, for 2 selected formulations, in vivo in healthy volunteers. Absorption in vivo comparing ointment and lipophilic cream was correlated with expectation based on the dermal delivery obtained in vitro. A 2.17-fold higher systemic exposure to BAY1003803 was achieved by the ointment formulation. This is well in line with the predicted exposure difference of 2.74 based on the in vitro data. In conclusion, in vitro skin absorption studies using human skin are suitable for the prediction of systemic exposure and formulation effects in vivo; they can therefore be applied to guide the design of clinical investigations of dermatological preparations.

Keywords

Clinical pharmacokinetics, lipophilic cream, ointment, skin penetration

Within the scope of a program to develop agents for the topical treatment of psoriasis,[1] we have investigated the compound BAY1003803, a novel nonsteroidal selective glucocorticoid receptor agonist.[2,3] Although clinical development of this substance has been discontinued because of inadequate efficacy, early-phase studies revealed some notable general findings concerning skin permeation, which we present here.

Recent investigations have demonstrated that percutaneous absorption studies in vitro may correlate with systemic exposure measured in clinical pharmacokinetic studies if the experimental conditions, such as dosing conditions, are well controlled.[4] Attempts have been made to discriminate between bioequivalent and nonbioequivalent dermal preparations on the basis of in vitro diffusion cell studies.[4,5] We therefore investigated whether in vitro skin absorption studies could also predict the difference in systemic absorption between different semisolid topical preparations.

The structure of BAY1003803 (5-{[(1S, 2S)1-(2-chloro-3-fluoro-4-methoxyphenyl)-3,3,3-trifluoro-2-hydroxy2-(methoxymethyl)propyl]amino}-7-fluoro-1H-quinolin-2-one) is shown in Figure 1. The major metabolic pathway of BAY1003803 in human microsomes and hepatocytes was O-demethylation to BAY1217469. Several cytochrome P450 (CYP) isoforms contribute to the formation of BAY1217469, with CYP3A4 being the most important. BAY1217469 binds also to the glucocorticoid receptor but with an

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Submitted for publication 4 July 2019; accepted 5 August 2019.

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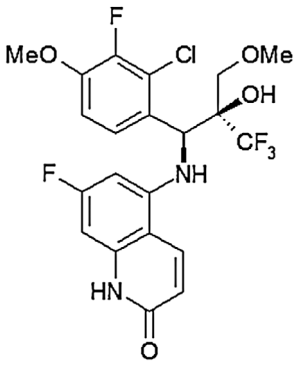

Figure 1. Structure of BAY1003803. The principal metabolite BAY1217469 is demethylated at the methoxy group shown here at upper right.

approximately 10-fold lower affinity as compared with the parent compound.

This neutral drug compound BAY1003803 is almost insoluble in water and has a calculated log P octanol/water of 3.3. Various semisolid formulation types were developed for dermal application and optimized with regard to pharmaceutical stability and drug delivery into the skin. An in vitro study was performed to assess the percutaneous absorption and distribution within the skin following topical application of [^{14}C]-BAY1003803 in 4 selected formulations to human skin. Two formulations were then selected for characterization in a clinical phase 1 study for safety and pharmacokinetics (PK) with dose/exposure escalation in each cohort based on predicted exposure. We then compared the human PK results with the predictions made on the basis of the in vitro experiment; the predictive value of the latter is assessed in this work.

The prediction was based on human in vitro data available for the estimation of dermal delivery and total amount of compound absorbed in each of the 2 formulation types. The systemic exposure after topical treatment is determined by the formulation type, which influences the penetration through the skin (currently derived from in vitro data on dermal delivery), the concentration of active drug in the formulation, the percentage of the body's surface area (BSA) treated, and the conditions of administration (occlusive, nonocclusive).

Materials and Methods

Formulations of BAY1003803

The following formulations were prepared containing 0.1% (w/w) BAY1003803:

- Ointment: The drug substance was dissolved in the hydrophilic phase of the ointment, which consisted of propylene glycol, propylene carbonate, hydroxypropylcellulose, carbomer copolymer type B, and sodium hydroxide. The hydrophilic phase was then dispersed in the lipophilic phase, which consisted of paraffins, white wax, and cyclomethicone.
- Hydrophilic cream: The drug substance was dissolved in propylene glycol and added to the hydrophilic phase of the cream formed by purified water, carbomer copolymer type B, and sodium hydroxide. Then the fatty phase, consisting of paraffins, was dispersed in the hydrophilic phase. The hydrophilic cream was physically unstable and therefore not selected for the present clinical study.
- Lipophilic cream: The drug substance was dissolved in the hydrophilic phase of the cream, which consisted of purified water, propylene glycol, magnesium sulfate, and polysorbate 80. The hydrophilic phase was then dispersed in the lipophilic phase, which comprised paraffins, white wax, and sorbitan sesquioleate.
- Milk (thin lotion): The drug substance was dissolved in the lipophilic phase of the milk, which consisted of medium-chain triglycerides, squalene, and macrogol 30 dipolyhydroxystearate. The hydrophilic phase, consisting of purified water, lactic acid, sodium lactate, magnesium sulfate, and glycerol, was then dispersed in the lipophilic phase. The milk formulation showed the lowest skin penetration in vitro and was therefore excluded from further investigation in clinical trials.

Skin Penetration In Vitro

The study was conducted according to the current practices for skin-penetration studies.[6] Full-thickness human skin samples (abdomen, breast, and/or back) were obtained from 6 donors, male and female, aged 30-51 years, from St John's Hospital (NHS Lothian, United Kingdom). The split-thickness skin was cut with an electric dermatome to a depth of 300-400 µm.

The skin (area 3.14 cm^2) was mounted in static diffusion cells (skin temperature 32 ± 1°C). The receptor fluid was phosphate-buffered saline containing bovine serum albumin (5% w/v), in which BAY1003803 is adequately soluble. Barrier integrity was confirmed by electrical resistance. Formulations containing [^{14}C]-BAY1003803 were applied in the donor chambers at an area dose of ~5 mg/cm^2 (N = 6 biological replicates; ie, each sample was from a different donor). Percutaneous absorption was assessed by collecting aliquots of receptor fluid before and 2, 4, 8, 12, 16, 20, 24, 30, 36, 42, and 48 hours after application. Forty-eight hours after application the skin was washed with commercial soap solution using a positive displacement pipette and dried with tissue-paper swabs. The skin samples

were removed and separated (stratum corneum using 20 adhesive tape strips, epidermis, upper and lower dermis). The apparatus (donor and receptor chamber) was rinsed in methanol to ensure complete recovery of [^{14}C]-BAY1003803. The radioactivity of all samples was measured by scintillation counting. All samples were counted together with representative blanks using a liquid scintillation analyzer (Packard 2100-TR, Packard Bioscience, Meriden, Connecticut) with automatic quench correction by external standard. Scintillation fluid (10 mL, Aquasafe 500 plus supplied by Zinsser Analytic, Eschborn, Germany) was added to the samples. Representative blank sample values were subtracted from sample count rates to give net disintegration per minute per sample. Before analysis, samples were allowed to stabilize with regard to light and temperature.

Radioprofiling was also performed. Separate control radioprofiling experiments were performed in vitro using fresh human skin to check that no metabolites were formed in skin (data and method description on file, not shown here); none were found, implying that any systemic metabolite found in vivo is formed systemically. This confirms that the metabolite BAY1217469 is formed hepatically.

Estimation of Human Systemic Exposure

An interspecies allometric scaling approach was applied based on rat and dog intravenous data, resulting in a predicted human clearance (CL) of 0.80 L/(h·kg).

The volume of distribution in humans could not be predicted by an interspecies scaling approach. However, following dermal application, drug absorption is usually slower than the elimination of the drug from the circulation. In consequence, the absorption half-life is substantially longer than the effective half-life of the drug ("flip-flop"). Therefore, only CL values, and not volume of distribution, are required to predict human exposure (area under the curve [AUC] and concentration at steady state) after dermal application.

In general, extent of exposure (AUC) can be predicted as the dermal delivered dose (D) divided by CL, where CL has been predicted by allometric scaling and D corresponds to the dermal delivery over 48 hours as determined in the in vitro percutaneous absorption study.

Clinical Study

This investigation was a double-blind, vehicle-controlled, single-dose escalation study conducted at a single center: CTC North, Hamburg, Germany.

Subjects were healthy men aged 18-64 years with a body mass index of 18-30 kg/m^2. Healthy skin and nonsmoker status (for at least 3 months) were required, along with consent to use adequate appropriate contraception. Key exclusion criteria included preexisting diseases that might affect the absorption, distribution, metabolism, and elimination of the study drug, known hypersensitivity to the active substances, excipients, or other materials used in the study, known severe allergies, and clinically relevant deviations of screened parameters from their normal ranges.

Three test products were investigated: lipophilic cream containing 0.01% BAY1003803, lipophilic cream containing 0.1% BAY1003803, and ointment containing 0.1% BAY1003803. The dose escalation, based on the predicted systemic exposure (AUC), was planned with dermal application to an increasing fraction of BSA. Each subject received a single 22-hour dermal occlusive application only. Each treatment cohort comprised 8 volunteers, of whom 6 were treated with verum and 2 with a placebo consisting of vehicle only. Four cohorts were treated:

- Lipophilic cream (0.01% BAY1003803 or placebo), 6.25% of BSA, 0.13 mg drug
- Lipophilic cream (0.01% BAY1003803 or placebo), 30% of BSA, 0.6 mg drug
- Lipophilic cream (0.1% BAY1003803 or placebo), 13% of BSA, 2.6 mg drug
- Ointment (0.1% BAY1003803 or placebo), 13% of BSA, 2.6 mg drug (Further cohorts using up to 60% of BSA were originally planned but were not investigated due to the discontinuation of clinical development of BAY1003803.)

Sites of administration were determined according to the Lund-Browder scheme,[7] and administration was occlusive, using kitchen-type plastic foil.

Blood samples for the PK analysis were taken before administration and 1.5, 3, 5, 7, 9, 11, 13, 15, 21, 22 (before removal of occlusive wrappings), 23, 24, 27, 31, 35, 39, and 47 hours after administration. Because of the PK behavior observed in cohort 3, for cohort 4 a 120-hour sample was taken instead of the 11-hour sample. The primary PK variables of BAY1003803 and its metabolite BAY1217469 in plasma were peak concentration (C_{max}), $AUC_{0\text{-tlast}}$, and $AUC_{0\text{-}22h}$; parameters were calculated by a model-independent, compartment-free method using WinNonlin (Version 5.3; Certara USA, Princeton, New Jersey).

Safety analysis comprised adverse events, safety laboratory values, vital signs, and electrocardiography. After treatment of each complete cohort, an interim safety assessment was carried out before proceeding to the next. Subjects' participation ended with a follow-up examination 120 hours after administration of BAY1003803.

Table 1. Distribution of [^{14}C]-BAY1003803 Following Topical Application to Human Skin in Various Formulations

Test Preparation	Ointment[a] 0.1 %, w/w	Hydrophilic Cream 0.1 %, w/w	Lipophilic Cream[a] 0.1 %, w/w	Milk 0.1 %, w/w
		Percentage of applied dose		
Dislodgeable dose 48 h	82.9 ± 3.93	63.4 ± 6.53	92.1 ± 2.03	86.0 ± 6.54
Stratum corneum tapes 1-2	0.96 ± 0.35	7.13 ± 3.09	0.55 ± 0.31	4.46 ± 3.35
Stratum corneum tapes 3-20	4.59 ± 2.10	11.7 ± 4.56	2.03 ± 0.65	5.38 ± 1.89
Unabsorbed dose	88.5 ± 2.52	82.3 ± 4.98	94.7 ± 2.29	95.9 ± 3.67
Absorbed dose	1.15 ± 0.55	1.53 ± 1.16	1.33 ± 0.36	0.33 ± 0.11
Dermal delivery	9.03 ± 2.97	15.0 ± 2.60	3.29 ± 1.60	1.60 ± 1.59
Potentially absorbable dose	13.6 ± 3.44	26.7 ± 4.72	5.32 ± 1.19	6.98 ± 2.91
Mass balance	97.5 ± 2.51	97.3 ± 3.71	98.0 ± 0.78	97.5 ± 2.96
		ng equivalents/cm^2		
Dislodgeable dose 48 h	4400 ± 209	3230 ± 333	4650 ± 103	4370 ± 332
Stratum corneum tapes 1-2	50.8 ± 18.5	363 ± 157	27.8 ± 15.4	227 ± 170
Stratum corneum tapes 3-20	244 ± 111	598 ± 232	102 ± 32.7	274 ± 96.0
Unabsorbed dose	4700 ± 134	4190 ± 254	4790 ± 116	4870 ± 186
Absorbed dose	61.1 ± 28.9	77.9 ± 59.3	67.1 ± 18.1	16.9 ± 5.51
Dermal delivery	483 ± 159	775 ± 135	166 ± 81.1	81.2 ± 81.0
Potentially absorbable dose	727 ± 182	1370 ± 246	269 ± 60.0	355 ± 148
Mass balance	5180 ± 135	4970 ± 190	4950 ± 39.4	4950 ± 150

Arithmetic means ± standard deviation are shown. Dislodgeable dose includes skin wash + tissue swabs + pipette tips + donor chamber wash. Unabsorbed dose includes dislodgeable dose + whole stratum corneum (all tape strips) + unexposed skin. Absorbed dose (percutaneous absorption) includes receptor fluid + receptor chamber wash. Dermal delivery includes epidermis + upper dermis + lower dermis + absorbed dose. Potentially absorbable dose includes dermal delivery + stratum corneum tapes 3-20. Mass balance includes dermal delivery + unabsorbed dose.
[a]Formulations used in clinical study.

Bioanalytical Assays of Clinical Samples

BAY1003803 and its metabolite BAY1217469 were determined in lithium heparin plasma after addition of internal standards ([$^2H_6$]-BAY1003803 and [$^{13}C_2$,$^2H_3$]-BAY1217469), by solid-phase extraction (Oasis HLB 30, Waters, Milford, Massachusetts) followed by liquid chromatography with tandem mass spectrometric detection. Chromatographic separation was achieved using a Kinetex (Phenomenex, Torrance, California) C18 column (100 × 2.1 mm, 1.7 µm particle size). The mobile phases consisted of 10 mmol/L ammonium formate in water (A) and acetonitrile (B). The flow rate was 0.65 mL/min with a gradient from 30% to 95% B. The temperature was set at 55°C, and the run time was ~5 minutes. The eluates were analyzed with a Sciex (Framingham, Massachusetts) API 6500+ tandem mass specrometric system with positive ion electrospray detection. Transitions monitored were 493.0 → 335.0 (BAY1003803), 499.0 → 335.0 ([$^2H_6$]-BAY1003803), 479.0 → 313.1 (BAY1217469), and 484.0 → 318.1 ([$^{13}C_2$,$^2H_3$]-BAY1217469). The calibration range of the procedure was from 5.0 (lower limit of quantification [LLOQ]) to 5000 ng/L for BAY1003803 and BAY1217469.

For BAY 1003803, mean interassay accuracy of back-calculated concentrations (except LLOQ) in calibrators ranged between 99.1% and 100.9%, and precision was ≤6.9%. Accuracy and precision at LLOQ were equal to 100.3% and 10.1%, respectively. Quality control samples in the concentration range from 15 to 4000 ng/L were determined with an accuracy of 99.8% to 102.7% and a precision of 4.7% to 7.3%.

For BAY1217469, mean interassay accuracy of back-calculated concentrations in calibrators ranged between 99.1% and 100.7%, and precision was ≤ 7.8%. Quality control samples in the concentration range from 15 to 4000 ng/L were determined with an accuracy of 99.3% to 101.3% and a precision of 5.5% to 11.5%.

Results

In Vitro Experiments

The results for the distribution of radioactivity, cumulative percutaneous absorption, and percutaneous flux for all test groups are provided in Table 1. Graphical representations of time profiles for cumulative percutaneous absorption of BAY10038303 are provided in Figure 2 (the metabolite BAY1217469 was not detected in these experiments). A summary of the results is presented in Table 2. Chromatographic radioprofiling of samples was performed. No substantial biotransformation was observed in the skin, and the radioactivity found for BAY1003803 represented >90% of the parent drug.

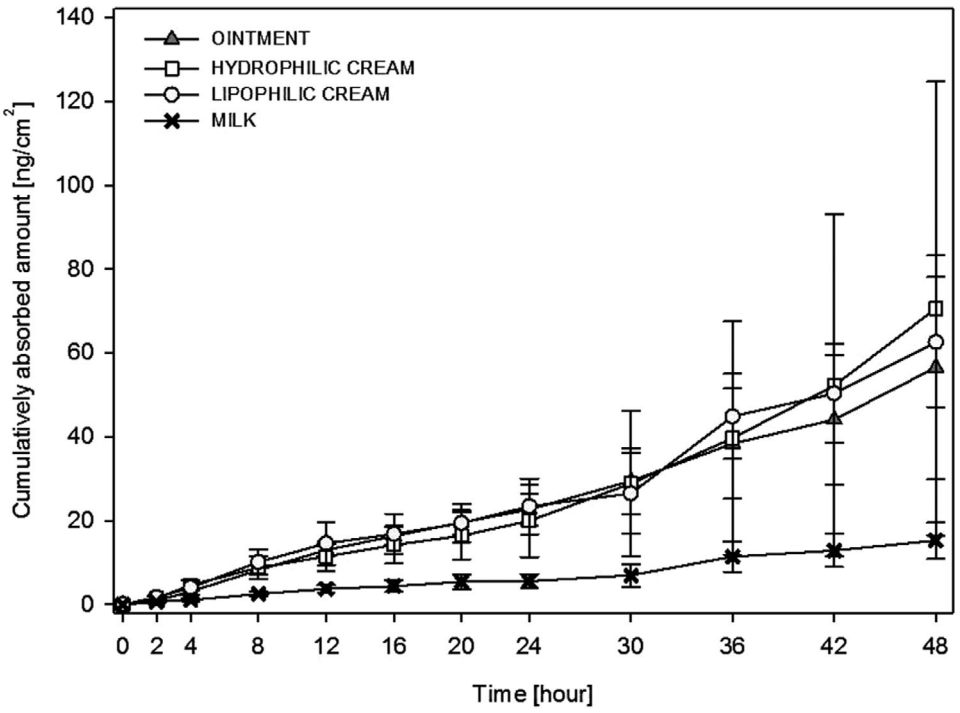

Figure 2. Percutaneous absorption of BAY1003803 in vitro. The plot shows the cumulative absorption (ng equiv/cm²) of [^{14}C]-BAY1003803 into receptor fluid following topical application of [^{14}C]-BAY1003803 in various test formulations (0.1%, w/w) to human split-thickness skin. Lipophilic cream and ointment were also tested in the clinical study. Arithmetic means and standard deviations are shown.

Table 2. Cumulative Percutaneous Absorption in ng-Equivalents per cm² of [^{14}C]-BAY1003803 Following Topical Application to Human Skin in Different Formulations

Test Preparation	Ointment[a]	Hydrophilic Cream	Lipophilic Cream[a]	Milk
Cumulative absorption 24 h	22.7 ± 3.8	20.0 ± 8.6	23.4 ± 6.6	5.60 ± 1.69
Cumulative absorption 48 h	56.6 ± 26.7	70.6 ± 54.2	62.6 ± 15.5	15.3 ± 4.3
Average flux 0-12 h	1.08 ± 0.11	0.96 ± 0.29	1.22 ± 0.43	0.31 ± 0.08
Average flux 12-24 h	0.81 ± 0.24	0.70 ± 0.49	0.74 ± 0.21	0.15 ± 0.10
Average flux 24-48 h	1.41 ± 0.98	2.11 ± 1.91	1.63 ± 0.46	0.40 ± 0.16
Maximum flux 24 h	1.45 ± 0.23	1.69 ± 0.58	1.75 ± 0.49	0.46 ± 0.12
Maximum flux 48 h	2.34 ± 1.76	3.26 ± 2.00	3.11 ± 0.87	0.75 ± 0.28

Arithmetic means ± SD are shown.
[a]Formulations used in clinical study.

Prediction of Human Exposure for the Formulations Clinically Investigated

The systemic drug exposure depends on the formulation type and the extent of the skin area treated. The systemic exposure was predicted relative to a dermally applied dose based on the in vitro dermal delivery data (Table 1) obtained with human skin and a drug clearance rate of 0.80 L/(h·kg) based on allometric scaling. The prediction considering a human body weight of 60 kg (CL 48 L/h) results in AUC of 1782 and 4891 ng·h/L, respectively, for groups 3 and 4.

Clinical Results: Subjects

Of 71 people screened, 32 were included in the study. The remaining 39 did not complete screening, in about one-half of cases (19) because the study was terminated. All 32 subjects who were included completed the study: 8 in each of 4 treatment cohorts, of whom 6 per cohort received active treatment and a total of 8 the vehicle placebo. The study population comprised 32 healthy men with a mean age of 41.2 years (range 19-62 years), mean weight 84.3 kg (range 59.0-102.2 kg), mean height 182 cm (range 167-196 cm), and mean

body mass index 25.5 kg/m^2 (range 18.6-29.6 kg/m^2). All subjects were white. Their mean total BSA was 2.1 m² (range 1.7-2.4 m²).

Clinical Results: Pharmacokinetics

The LLOQ for BAY1003803 and for its metabolite BAY1217469 in human plasma is 5 ng/L. Most plasma concentrations measured in cohorts 1 and 2 were below this for both substances, so no PK evaluation could be performed for these 2 cohorts.

For cohort 3, arithmetic mean plasma concentrations of BAY1003803 increased, in particular during the second day after initial exposure, with C_{max} being reached after approximately 33 hours. Individual times to C_{max} (t_{max}) were broadly distributed (31-39 hours). The first blood samples for PK, taken 1.5, 3, 5, 7, 11, and 13 hours after the start of administration, all had concentrations below the LLOQ (see Figure 3).

The late t_{max} was unexpected; therefore, after this had been observed for cohort 3, a late (120 hours) PK sample was introduced for dose cohort 4. The late C_{max} and the persistent plasma levels after 120 hours were also observed in cohort 4. In consequence of the late t_{max}, the planned PK parameter $AUC_{0\text{-}22}$ has limited relevance. Another unanticipated observation was that in some subjects there seemed to be an increase in exposure shortly after removal of the occlusive wrappings and having a shower 22 hours after the start of administration of the drug.

The metabolite BAY1217469 could be quantified for cohorts 3 and 4 (see Figure 3). The concentration-time profiles qualitatively resembled those for the parent compound.

PK parameters are summarized in Table 3 for cohorts 3 and 4 (as stated, analysis was not possible for cohorts 1 and 2). It is to be noted that t_{last} was increased for cohort 4 (see above); this will have affected the corresponding AUC(0–t_{last}) value. Therefore, to facilitate comparison between the cohorts, AUC(0–47) was calculated for both cohorts 3 and 4. For dose normalization for values derived from cohorts 3 and 4 (see Figure 4), it should be noted that the same dose (2.6 mg BAY1003803) was applied on 13% of BSA for subjects in both of these cohorts.

Clinical Results: Safety

No serious or severe adverse events occurred in the study. No subject discontinued the study prematurely because of an adverse event. There were no treatment-emergent adverse events (TEAEs) related to the study drug, and all adverse events resolved. Among the 24 actively treated subjects, 7 subjects reported a total of 9 TEAEs (in cohort 1, 3 subjects with 3 events; in cohort 3, 2 subjects with 2 events; in cohort 4, 2 subjects with 4 events; for treatments see Methods/Clinical Study). Among the 8 vehicle-treated subjects, 3 subjects reported a total of 5 TEAEs.

Six of the 14 TEAEs were related to protocol-required procedures, mainly skin/cutaneous TEAEs (1 event of erythema, 1 of blisters, and 2 of skin abrasion among drug-treated subjects and 1 of erythema in a placebo-treated subject). There was 1 vascular TEAE (thrombophlebitis, in a placebo-treated subject). Among the drug-treated subjects 5 of the TEAEs reported were mild and 4 of moderate severity; among the placebo-treated subjects the corresponding numbers were 3 and 2, respectively. The outcomes of all TEAEs were documented as "resolved/recovered" at the end of the study.

Discussion

In the treatment of dermatological indications such as psoriasis, local drug levels in the skin are related to clinical efficacy.[8,9] However, the presence of systemic drug may lead to systemic adverse effects.[10] For BAY1003803, local anti-inflammatory effects need to be balanced against adverse suppression of the hypothalamic-pituitary axis, resulting in reduced cortisol levels. In consequence, an optimum formulation would provide high drug levels in the skin but low systemic bioavailability.

In a series of experiments in vitro, several formulations were pretested. Four promising candidates were investigated in this work with radiolabeled BAY1003803 to allow characterization of skin absorption, metabolism, distribution, and elimination. All formulations selected for clinical PK investigation were considered to have a chance of successful drug delivery to the skin; the milk formulation was not included in the clinical trial because it showed lowest drug delivery into the skin, and the hydrophilic cream was not included in the clinical trial because it was physically unstable in further CMC characterizations.

Before the start of clinical trials, a risk assessment was performed considering local and systemic tolerability. The starting dose was derived by considering the systemic exposure observed at the dose reflecting the preclinical no-adverse-event level for systemic effects. The systemic exposure in humans was predicted by taking both drug absorption and drug clearance into account. In particular, predicted in vivo clearance by allometric scaling and percutaneous absorption in vivo by human skin absorption in vitro were considered.

An in vitro–versus–in vivo comparison of the PK results was performed. On the basis of the results obtained in vivo it was assessed whether the extent of exposure was correctly predicted, the time course of absorption was reflected by the in vitro experiments, and the differences in performance between the

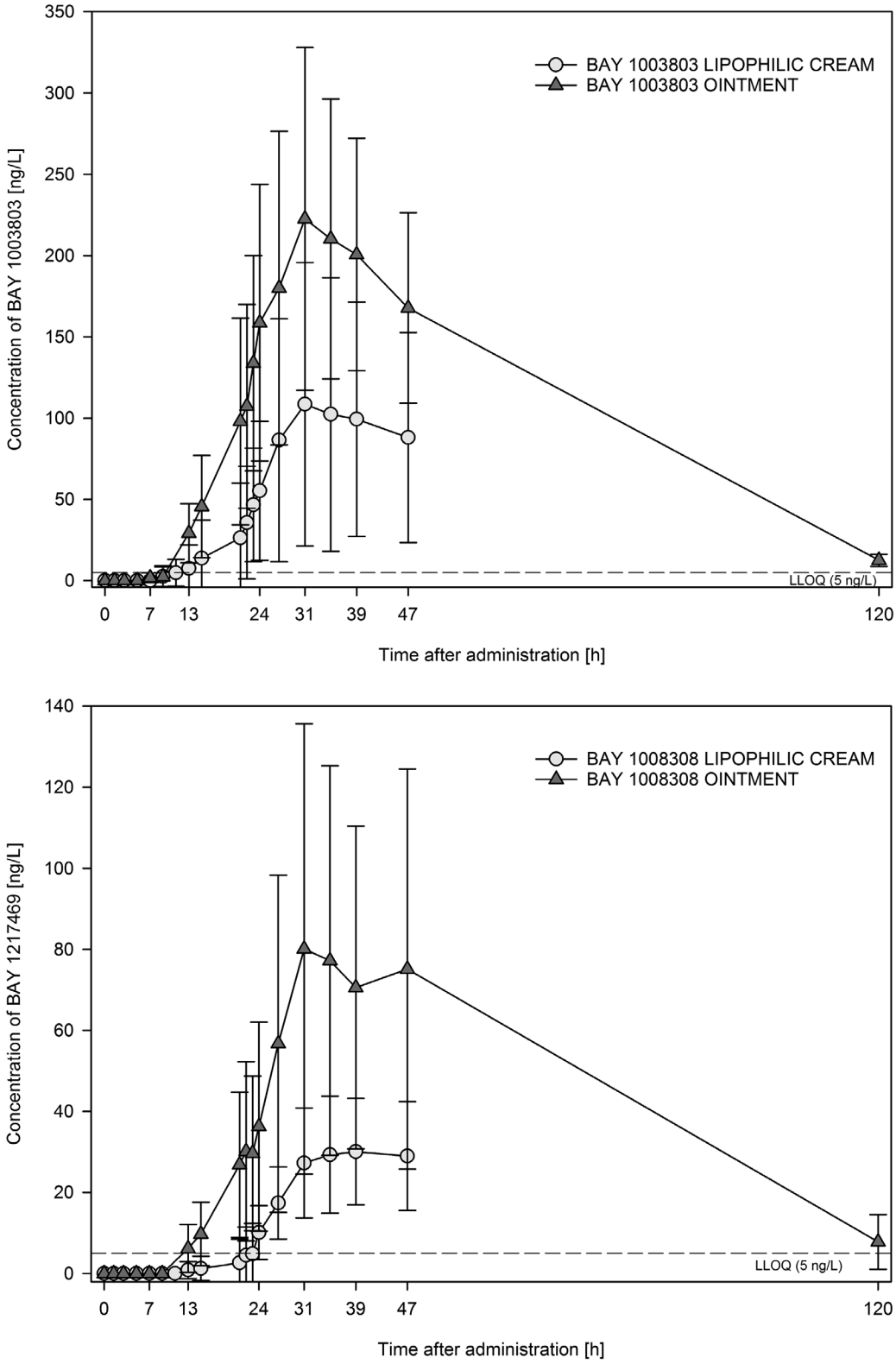

Figure 3. Plasma concentrations of BAY1003803 (top) and BAY1217469 (bottom) following 22 hours of administration of BAY1003803. In the upper diagram the arithmetic mean concentrations of BAY1003803 after administration are shown. Upper curve, cohort 4 (ointment, 0.1% BAY1003803, 13% of BSA); lower curve, cohort 3 (lipophilic cream, 0.1% BAY1003803, 13% of BSA). Arithmetic means and standard deviations are shown. In the lower diagram the corresponding concentrations of the metabolite BAY1217469 are shown. LLOQ (5 ng/L for each substance) indicates lower limit of quantification. Planned sampling times are plotted.

Table 3. Summary Statistics of Observed Pharmacokinetic Parameters of BAY1003803 and Its Metabolite BAY1217469 in Plasma for Cohorts 3 and 4 of the Clinical Study

		BAY1003803 0.1% Lipophilic Cream, 13% BSA	BAY1003803 0.1% Ointment, 13% BSA
		BAY1003803 (parent compound)	
C_{max}	ng/L	112 ± 87.2	232 ± 95.9
AUC_{0-22}	ng·h/L	405 ± 321	681 ± 453
AUC_{0-47}	ng·h/L	2492 ± 2032	5417 ± 2416
$AUC_{0-tlast}$[a]	ng·h/L	2501 ± 2044	9720 ± 3533
AUC	ng·h/L	NA	10,403 ± 3830
t_{max}	h	33.0 (31.0-39.0)	33.3 (31.0-39.0)
		BAY1217469 (main metabolite)	
C_{max}	ng/L	31.7 ± 13.7	84.4 ± 52.8
AUC_{0-47}	ng·h/L	634 ± 319	1834± 1168
$AUC_{0-tlast}$[a]	ng·h/L	636 ± 321	3674 ± 2766
t_{max}	h	37.0 (31.0-47.0)	35.0 (31.0-47.0)

BSA indicates body surface area; NA, not available.
For C_{max} and AUC arithmetic means and standard deviations are shown, with median and range for t_{max}.
[a]t_{last} was 47 and 120 hours for cohorts 3 and 4, respectively (see text).

formulations in vitro (dermal delivery and potentially absorbed dose; see Table 1) and in vivo (human exposure; see Table 3) were consistent.

In vitro, a comparison of cumulative percutaneous absorption for the 4 different formulations indicated that cumulative percutaneous absorption (ng equiv/cm^2) and maximum flux (ng equiv/cm^2 per hour) of [^{14}C]-BAY1003803 were highest for the ointment and cream formulations, where similar percutaneous absorption and flux values were observed. The hydrophilic cream was found to yield a very high flux, so that ointment and lipophilic cream were selected for the clinical study. The total absorbed dose amounted to approximately 61 and 67 ng equiv/cm^2 in the ointment and lipophilic cream test group, respectively. The lowest cumulative percutaneous absorption and maximum flux for [^{14}C]-BAY1003803 were observed with the milk formulation, for which the total percutaneous absorption was 16.9 ng equiv/cm^2. Because the flux results were variable for some samples and test groups, cumulative percutaneous absorption was considered more reliable for comparison purposes.

A comparison of dermal delivery for the 4 different formulations indicated that dermal delivery of [^{14}C]-BAY1003803 was lowest for the milk (81 ng equiv/cm^2) and highest for the hydrophilic cream (775 ng equiv/cm^2), ie, higher by a factor approaching 10.

A comparison of potentially absorbable dose for the various formulations indicated that potentially absorbable dose of [^{14}C]-BAY1003803 was highest for the hydrophilic cream (1370 ng equiv/cm^2). The lowest potentially absorbable dose for [^{14}C]-BAY1003803 was observed with the lipophilic cream (269 ng equiv/cm^2).

There was no obvious degradation of [^{14}C]-BAY1003803 in the samples investigated; specifically, the known systemic main metabolite BAY1217469 was not detected.

In summary, the in vitro experiments with the 4 formulations assessed led to the following conclusions based on observed absorption profile, percutaneous flux, dermal delivery, and potentially absorbable dose for [^{14}C]-BAY1003803 through human skin:

- The hydrophilic cream showed the highest dermal delivery. This formulation, however, was physically unstable in further CMC characterizations and thus not further clinically tested.
- The milk formulation showed the lowest dermal delivery and percutaneous absorption and was therefore not selected for further clinical investigation, as this formulation was expected to provide the least clinical benefit.
- The ointment and lipophilic creams showed high dermal delivery, which justified further clinical evaluation of both formulations.

The clinical study was terminated early because of results from a parallel clinical trial investigating efficacy parameters in the psoriasis plaque test, so only limited clinical data were obtained. Dose and exposure escalation were performed as planned for the 4 cohorts, and no safety signals were detected that might have suggested poor tolerability of BAY1003803 administered as lipophilic cream (0.01% and 0.1%) or as ointment (0.1%) for 22 hours under occlusion. There were no severe or serious TEAEs.

Exposure data and PK parameters for BAY1003803 could be estimated for dose cohorts 3 and 4. This nonetheless allows direct comparison, as the same BSA was treated and the same amount of BAY1003803 (2.6 mg) was applied, and its metabolite BAY1217469 was detected in these dose cohorts, confirming the formation of the latter in vivo (most probably in the liver).

As expected, a higher systemic exposure to BAY1003803 was achieved by the ointment formulation. The calculated arithmetic mean AUC_{0-47} was 2492 and 5417 ng·h/L for dose cohorts 3 and 4, respectively. The ratio of these values is 2.17, which is well in line with the predicted exposure ratio of 2.74 (4891/1782). Therefore, the strategy with regard to dose escalation using the exposure predicted by considering the potentially absorbed dose obtained in vitro and the BSA treated in vivo is confirmed. This approach proved also to be suitable for predicting

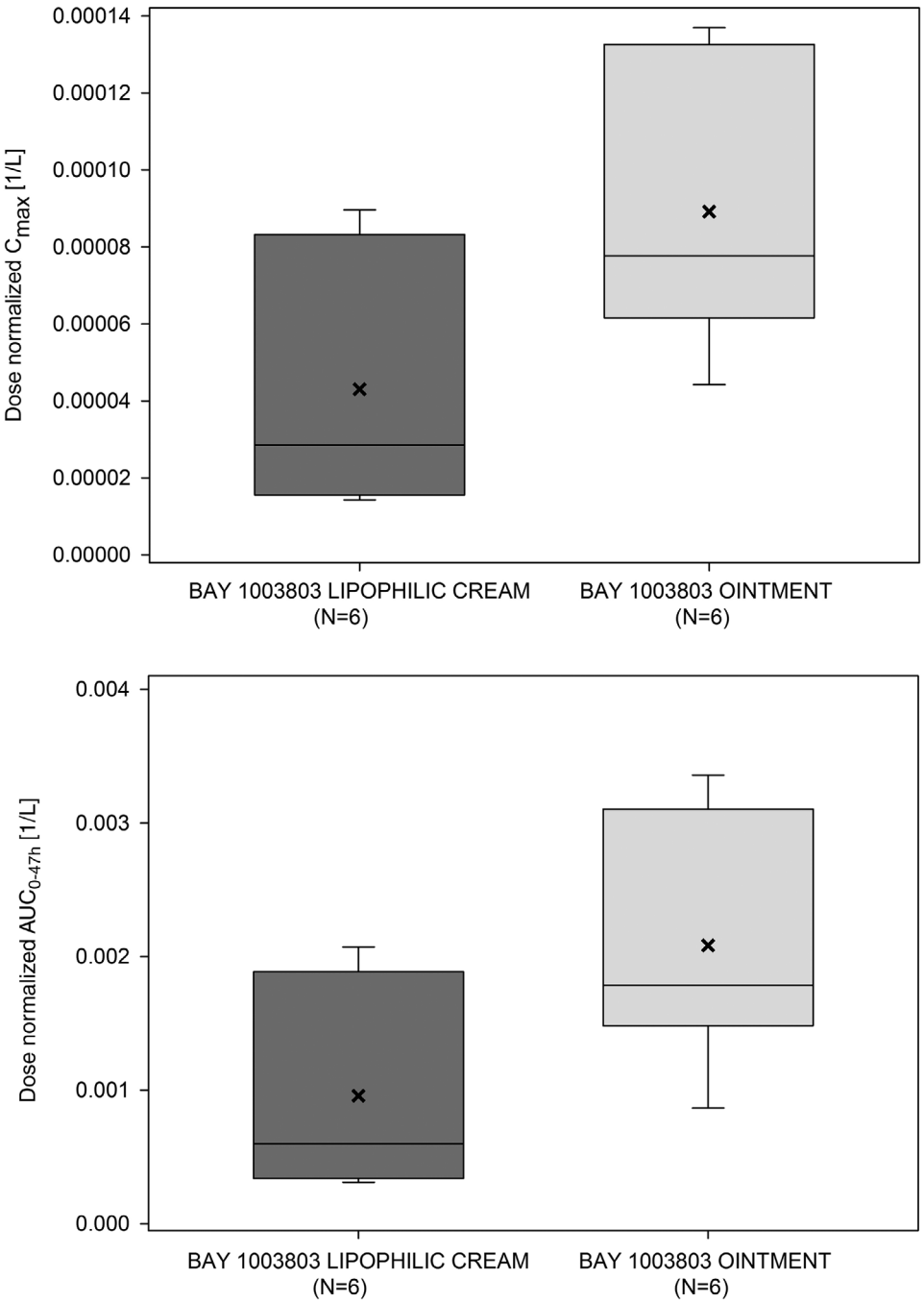

Figure 4. Box plots for C_{max}/D (top) and $AUC_{0–47}$/D (bottom) of BAY1003803 in plasma. Box illustrates 25th to 75th percentiles; horizontal line, median; whiskers, minimum and maximum values; cross, arithmetic mean. AUC_{0-47} indicates area under the concentration-time curve from 0 to 47 hours; C_{max}, peak concentration.

formulation-dependent differences in systemic exposure. The predicted BAY1003803 AUC, based on the in vitro dermal delivery, BSA treated, applied area dose, and the estimated clearance rate, was 1782 ng·h/L and 4891 ng·h/L for the ointment 0.1% and lipophilic cream 0.1%, respectively. The closest AUC obtained from the in vivo clinical comparison (AUC_{0-47}) was 2492 and 5417 ng·h/L for dose cohorts 3 and 4, respectively. Therefore, this was 1.40 and 1.11 times the predicted value, respectively. Whereas applied area dose, BSA treated, and clearance rate were not influenced by the formulation, only the parameter "dermal delivery" reflected the different formulation effect on drug absorption and systemic exposure. Thus, the dermal delivery obtained in vitro corresponded closely with the in vivo exposure.

It was expected that the greatest part of the systemic exposure would be observed on the day of administration, as the lag time obtained (in vitro) for various compounds of different lipophilicity is usually rather short.[11] However, this was not the case (Figure 3): the $AUC_{0\text{-}22}$ reflects only a very small portion of the overall AUC, for example, less than 7% for dose cohort 4 (681/10,403, Table 3). Therefore, $AUC_{0\text{-}22}$ is less meaningful. The reason for the slow absorption, with a median t_{max} of ~33 hours, is not known. However, it is known that the stratum corneum and pilosebaceous units are compartments where lipophilic drugs can be stored for up to 2 weeks and probably even longer.[12,13] Whereas in vitro skin fluxes were relatively constant between 4 and 24 hours after dosing, a substantially prolonged lag time of >10 hours was observed in vivo. It may be noted, however, that the maximum and average flux in vitro between 24 and 48 hours is higher than those in the first 24 hours. Therefore, this in vitro observation may have been indicative for the in vivo finding of a slow absorption process resulting in a late C_{max}. Furthermore, it has been shown for caffeine and testosterone that t_{max} in vivo is substantially longer than for the fast in vitro percutaneous absorption. In addition, t_{max} can be substantially influenced by the choice of vehicle.[14]

Despite the slow permeation of BAY1003803 through the skin and the low systemic exposure during the first 22 hours, systemic exposure was in the range predicted from preclinical data. The strategy of performing escalation (in particular from dose cohorts 3 to 4) by using a fixed amount of drug and a fixed percentage of BSA to compare 2 different formulations was confirmed.

In this single-dose escalation study using different formulations of BAY1003803, the predicted exposure was overall in agreement with the observed drug plasma levels. The observed AUC of BAY1003803 in cohort 4 (9720 ng·h/L) is approximately 2.0 times the predicted AUC (4891 ng·h/L). A factor of 2 between predicted and in vivo measured CL rates based on scaling models is widely accepted[15]; in particular, the route of administration should be noted, as for orally administered drugs, more experience and confidence for prediction are available. As the predicted human CL is also used to predict the systemic exposure after dermal application, the same predictive power needs to be accepted for the systemic exposure estimate after dermal application as well.

We note that a similar scheme of formulation comparison, ie, by skin penetration in vitro, was recently used to demonstrate bioequivalence, and the results of the comparison were accepted by the FDA in connection with the marketing authorization for Lotrimin Ultra cream.[16]

Conclusion

Our results indicate that in vitro skin absorption studies using human skin are suitable for the prediction of systemic exposure of various formulations in vivo; they can therefore be applied to guide the design of a clinical investigation of dermatological preparations.

Acknowledgment

The authors thank Antonia Kohnke for PK analysis, Joachim Höchel and Beate Rohde for discussion during manuscript preparation, and Paul Woolley for assistance with the language in this article.

Statement of Ethics

The clinical study has been conducted at CTC North GmbH and Co, KG in Hamburg, Germany. The clinical study protocol (EudraCT number 2016-000964-41) was approved by the Ethics Committee of the Landesärztekammer Hamburg and performed in accordance with the ethical standards of the Declaration of Helsinki and Good Clinical Practice. The written informed consent of all participants was obtained before any study procedures were performed.

For the in vitro work performed at Charles River Laboratories Edinburgh Ltd (UK), before surgery each patient provided informed consent for his skin to be taken for scientific purposes in accordance the ethical approval for receipt and the use of human skin of the NHS trust. Ethical approval for receipt and the use of human skin was obtained from Lothian Local Research Ethics Committee (REC Reference No. 06/S1101/19).

Conflicts of Interest

The authors are employees of the respective institutions to which they are affiliated. C.G., K.K., T.S., and R.N. own employee shares in Bayer AG.

Funding Sources

Both studies (preclinical in vitro and clinical) were funded entirely by Bayer AG.

Author Contributions

All listed authors meet the criteria for authorship and contributed significantly to the study concepts. All authors were involved in the analysis and interpretation of the data as well as in the manuscript preparation.

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