

Fixed-Combination Gels of Adapalene and Benzoyl Peroxide Provide Optimal Percutaneous Absorption Compared to Monad Formulations of These Compounds: Results from Two In Vitro Studies

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ABSTRACT

Introduction: Adapalene 0.1%/benzoyl peroxide 2.5% (0.1% A/BPO) and adapalene 0.3%/BPO 2.5% (0.3% A/BPO) gels are fixed-combination options for the topical treatment of acne. However, the active compounds of these combinations are also available as monads, to be used in association or as monotherapy. These two in vitro studies determined the effect of different treatment regimens on the percutaneous absorption of adapalene (0.1% and 0.3%) gels and BPO 2.5% gel in ex vivo human skin.

Methods: In vitro percutaneous absorption studies were conducted using full-thickness human skin from six donors. Treatment regimens included the application of 0.1% A/BPO, 0.3% A/BPO, or four free-combination regimens of the monads. Skin samples were

incubated for 24 h. Concentrations of adapalene and BPO equivalent (BPO-eq) (i.e. benzoic acid after chemical transformation of BPO) were measured using high-performance liquid chromatography. Comparison of regimens was performed using a bioequivalence criterion (estimated ratio between 0.8 and 1.25).

Results: The fixed combination 0.3% A/BPO regimen demonstrated more than three times higher absorption of adapalene versus the fixed-combination 0.1% A/BPO. Based on the bioequivalence acceptance criterion, all four free-combination regimens were different from 0.1% A/BPO and 0.3% A/BPO, with higher adapalene release delivered by the fixed combinations versus the free combinations. For BPO-eq, the results showed that the free-combination regimens where adapalene 0.1% was applied first were different from 0.1% A/BPO, with lower BPO-eq release delivered by these regimens compared to the fixed combination. The regimen adapalene 0.3% for 10 h followed by BPO 2.5% delivered lower BPO-eq release compared to the fixed combination.

Conclusion: The fixed-combination A/BPO gels provide optimal percutaneous absorption of the

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active compounds compared to free combinations of adapalene 0.1%, adapalene 0.3%, and BPO 2.5%. The higher concentration of adapalene in the 0.3% A/BPO gel and the resulting higher absorption may explain higher clinical efficacy.

Keywords: Adapalene/benzoyl peroxide gel; Fixed combination; In vitro; Optimal percutaneous absorption

INTRODUCTION

Acne is a chronic disease which results in significant psychological burden and reduced quality of life for patients [1]. The disease has been ranked as the eighth most prevalent disease worldwide [2]. Prompt and effective treatment is crucial for the prevention of acne sequelae [3]. The current armamentarium for the topical treatment of acne includes an array of agents which can be used in combination [4]. A combination recommended by international guidelines for the topical treatment of acne involves the use of a retinoid, such as adapalene, with benzoyl peroxide (BPO) [4–6].

Adapalene possesses comedolytic, anticomedogenic, and anti-inflammatory properties [7–11]. The efficacy and safety of adapalene 0.1% gel has been demonstrated in clinical studies [10–12]. Further studies have reported that a higher concentration of the formulation (adapalene 0.3% gel) shows superior efficacy and a similar tolerability profile compared to adapalene 0.1% gel and vehicle [13, 14]. Clinical data have also demonstrated that adapalene 0.3% gel was safe and effective in the long-term (12 months) treatment of patients with acne [15]. BPO is the antimicrobial agent of choice due to its bactericidal properties against *Propionibacterium acnes* (*P. acnes*), and absence of

antibiotic resistance [16–18]. Formulations of both active compounds are available as monads, to be used in association or as monotherapy for the treatment of acne.

Adapalene 0.1%/BPO 2.5% gel (Epiduo®; Galderma, Lausanne, Switzerland) (0.1% A/BPO) is an antibiotic-free, fixed-dose combination of adapalene and BPO, which is efficacious in mild-to-moderate acne [16–19]. Data from randomized clinical studies demonstrated that 0.1% A/BPO provided synergistic and significantly greater efficacy than its monads in the treatment of acne, with an acceptable safety profile [17–21]. Recent clinical evidence, demonstrated the significantly greater efficacy of higher concentration adapalene 0.3%/BPO 2.5% fixed-dose topical gel (Epiduo® Forte; Galderma) (0.3% A/BPO) compared to vehicle as well as a good safety profile in patients with moderate-to-severe acne, increasing treatment options [22]. Although it has been demonstrated that the fixed combination can provide superior success in the reduction of acne lesions and an improvement in the clinical condition of patients compared to that of its monads [16–21], there are no data on the drug bioavailability and bioequivalence of these agents. In vitro percutaneous absorption studies could be used for the comparison of these treatment regimens [23, 24].

The objective of these two studies was to determine the effect of different treatment regimens on the percutaneous absorption of adapalene (0.1% and 0.3%) gel and BPO 2.5% gel in ex vivo human skin.

METHODS

In vitro percutaneous absorption studies were conducted using full-thickness human skin

from three donors (per study), mounted on polycarbonate membrane inserts of 6-well culture plates. Skin samples of about 2×2 cm were cut out from the isolated skin after careful removal of subcutaneous fat. Skin samples were then carefully transferred into six-well plates on a Transwell[®] insert which allows contact of the receptor fluid to the dermal side of the skin sample, while the *stratum corneum* remains exposed to the air. Glass cylinders of 1 cm^2 surface were glued to the epidermal side of the skin sample using cyanoacrylate-based glue. This keeps the skin flat and in constant contact with the receptor liquid and prevents bending.

A dose of 10 mg/cm^2 of each formulation was applied on the skin surface (application area of 1 cm^2) and 2 mL of phosphate buffer saline were added in the receptor compartment. A dose of 10 mg/cm^2 was chosen taking into account the clinical use conditions (1–5 mg/cm²) and the sensitivity of analytical methods.

The 0.1% A/BPO study included the following treatment regimens:

- 0.1% A/BPO gel
- BPO 2.5% gel for 10 min followed by adapalene 0.1% gel.
- Adapalene 0.1% gel for 10 min followed by BPO 2.5% gel.
- BPO 2.5% gel for 10 h followed by adapalene 0.1% gel.
- Adapalene 0.1% gel for 10 h followed by BPO 2.5% gel.

The 0.3% A/BPO study included the following treatment regimens:

- 0.3% A/BPO gel
- BPO 2.5% gel for 10 min followed by adapalene 0.3% gel.
- Adapalene 0.3% gel for 10 min followed by BPO 2.5% gel.
- BPO 2.5% gel for 10 h followed by adapalene 0.3% gel.

- Adapalene 0.3% gel for 10 h followed by BPO 2.5% gel.

The sequential application of monads separated by 10 min was chosen in order to simulate the use of both monads in association at the same time by the patient. The sequential application of monads separated by 10 h was chosen in order to simulate the use of monad as monotherapy. This interval was chosen to mimic morning/evening application of each monad in patient use conditions while respecting technical and operational constraints of the ex vivo test.

Skin samples were incubated (37 °C and 5% CO₂) for 24 h. Each condition was performed in triplicate for each of the three donors (i.e. $n = 9$). Concentrations of adapalene and BPO equivalent (BPO-eq) (i.e. benzoic acid after chemical transformation of BPO) were measured using high-performance liquid chromatography.

Comparison between the different treatment regimens was performed using the geometric mean ratio of test/reference treatment approach a (bioequivalence criterion estimated ratio of geometric means between 0.8 and 1.25).

Compliance with Ethics Guidelines

This article does not contain any new studies with human or animal subjects performed by any of the authors.

RESULTS

1% A/BPO Study

Skin Characteristics

Three full-thickness skin samples from three female donors, aged 56–79 years, were used in the study. The skin sample thickness ranged between 1.7 and 2.2 mm.

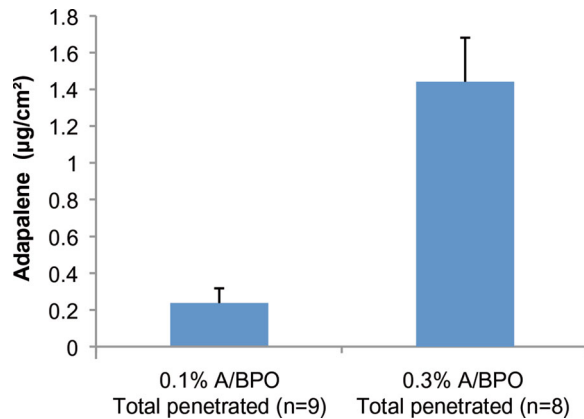


Fig. 1 Adapalene: total penetrated into skin ($\mu\text{g}/\text{cm}^2$) (mean and standard error of the mean)

Adapalene Release

Figure 1 illustrates the total penetrated adapalene for 0.1% A/BPO. Based on the bioequivalence acceptance criterion, the results showed that all free-combination regimens were different from 0.1% A/BPO gel, with geometric mean ratios outside the acceptance interval of 80–120% (Fig. 2). The 0.1% A/BPO gel showed higher adapalene release compared to all monad formulations.

BPO-eq Release

Figure 3 illustrates the total penetrated BPO-eq for 0.1% A/BPO. According to the bioequivalence

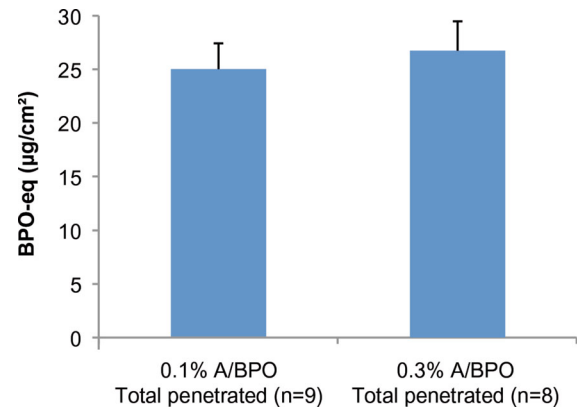


Fig. 3 BPO-eq: total penetrated into skin ($\mu\text{g}/\text{cm}^2$) (mean and standard error of the mean)

acceptance criterion, the free-combination regimens with application of adapalene 0.1% gel prior to BPO 2.5% gel were different from A/BPO gel treatment (Fig. 4). The 0.1% A/BPO gel showed higher BPO-eq release compared to monad formulations.

3% A/BPO Study

Skin Characteristics

Three full-thickness skin samples from 2 male and 1 female donors, aged 31–55 years, were used in the study. The skin sample thickness was between 1.6 and 3.2 mm.

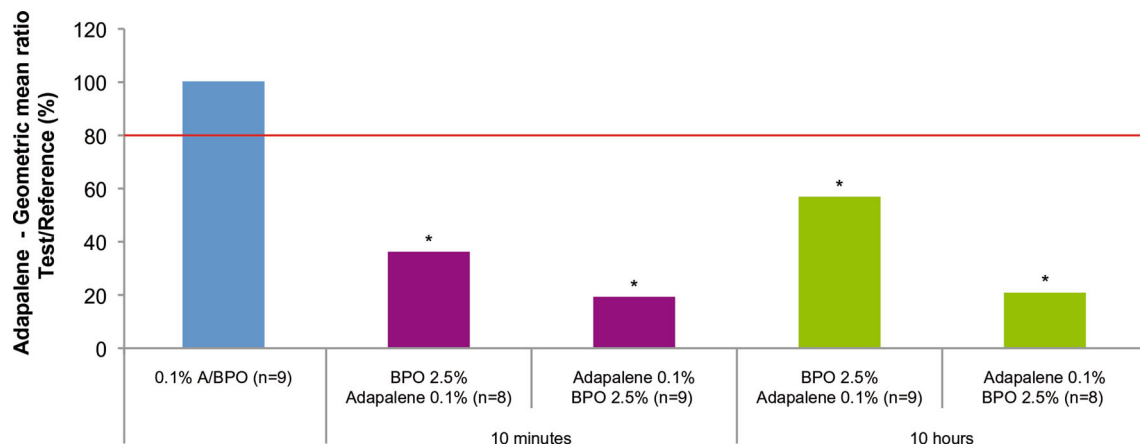


Fig. 2 Adapalene: comparison between treatment regimens (geometric mean ratio, %). Asterisk outside the acceptance interval of 80–125% (<80%)

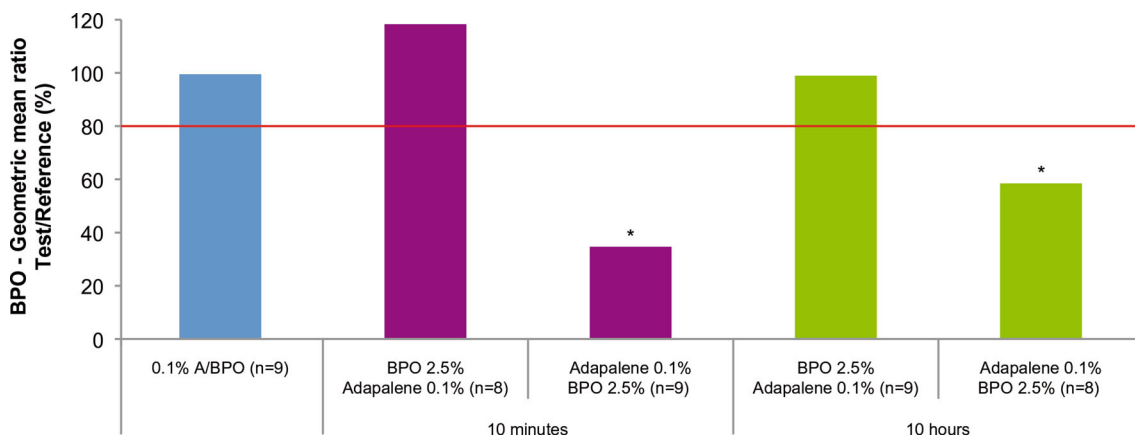


Fig. 4 BPO-eq: comparison between treatment regimens (geometric mean ratio, %). *Asterisk* outside the acceptance interval of 80–125% (<80%)

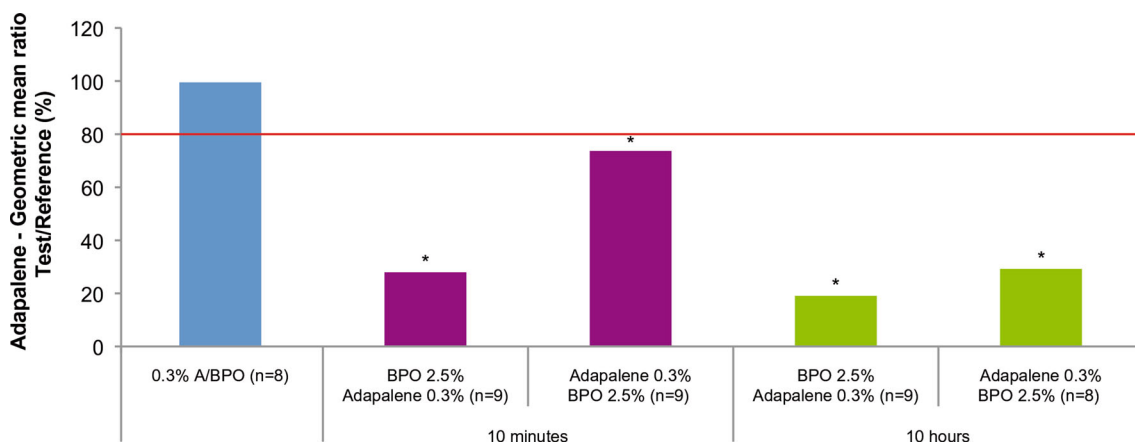


Fig. 5 Adapalene: comparison between treatment regimens (geometric mean ratio, %). *Asterisk* outside the acceptance interval of 80–125% (<80%)

Adapalene Release

Figure 1 illustrates the total penetrated adapalene for 0.3% A/BPO. Skin penetration of adapalene in 0.3% A/BPO gel was more than three times higher than in 0.1% A/BPO gel. This may be explained in part by the sensitivity of the limits of analytical method that may underestimate the concentration of adapalene in 0.1% A/BPO gel.

Based on the bioequivalence acceptance criterion, the results showed that all free-combination regimens were different from

0.3% A/BPO (Fig. 5). The fixed-combination 0.3% A/BPO gel showed higher adapalene release compared to all free combinations of monad formulations.

BPO-eq Release

Figure 3 illustrates the total penetrated BPO-eq for 0.3% A/BPO. The regimen adapalene 0.3% for 10 h followed by BPO 2.5% showed lower BPO-eq release when compared to that observed for 0.3% A/BPO (Fig. 6).

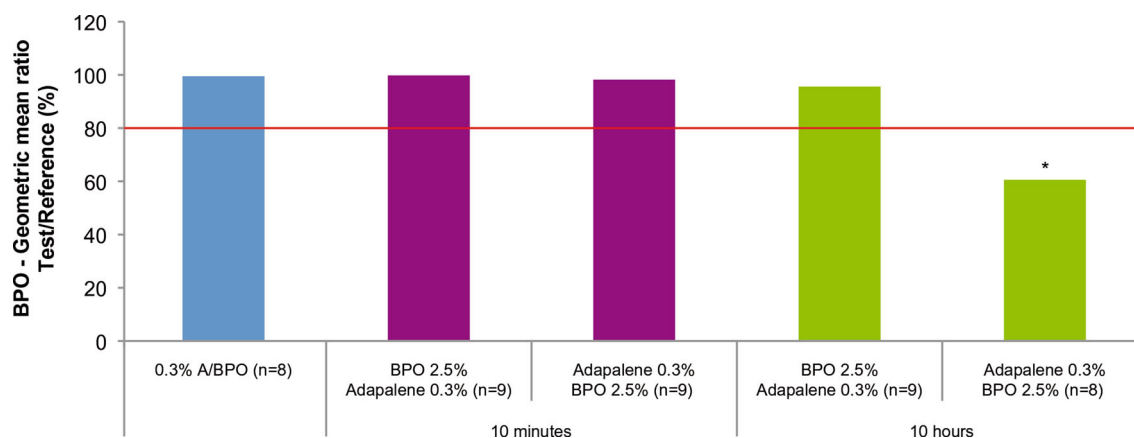


Fig. 6 BPO-eq: comparison between treatment regimens (geometric mean ratio, %). *Asterisk* outside the acceptance interval of 80–125% (<80%)

DISCUSSION

The studies described in this article investigated the effect of different treatment regimens on the percutaneous absorption of adapalene (0.1% and 0.3%) gel and BPO 2.5% gel in ex vivo human skin.

Comparison between the different treatment regimens was performed using the geometric mean ratio of test/reference treatment approach, since quantities recovered in the skin in this kind of experiment generally show a distribution skewed to the right and logarithmic transformation of the data normalizes the distribution (making it symmetrical). Moreover, a geometric mean of different ratios has the advantage of being the same as the ratio of different geometric means, a property that arithmetic means do not have.

Comparison of the two fixed combinations demonstrated more than three times higher absorption of adapalene in the 0.3% A/BPO regimen versus 0.1% A/BPO. In previous clinical studies on adapalene, it was demonstrated that 0.3% A/BPO gel resulted in higher efficacy compared to 0.1% A/BPO in the treatment of severe inflammatory acne [22, 25]. The higher concentration of adapalene in the 0.3% A/BPO

gel and the resulting higher absorption may explain the higher clinical efficacy. Moreover, the higher concentration of adapalene did not lead to lower safety since both 0.3% A/BPO and 0.1% A/BPO were safe and well tolerated [25].

The total amount of penetrated adapalene was higher after the fixed combinations 0.1% A/BPO and 0.3% A/BPO compared to that of the free-combination regimens. The geometric mean ratios were all out of the acceptance interval of bioequivalence limits (80–125%), indicating that all four free-combination regimens were different from 0.1% A/BPO and 0.3% A/BPO, with higher adapalene release delivered by the fixed combinations versus any of the other application regimens. The low level of adapalene in the free-combination regimens may be due to the creation of a physical barrier on the *stratum corneum* by BPO combined with the slow release of adapalene.

The results of clinical studies indicated that fixed combination of A/BPO is more advantageous compared to the free combinations of its monads in terms of efficacy and patient satisfaction [17–21]. Our in vitro results showing higher release of adapalene in the fixed combination than in its monads may explain the higher clinical efficacy.

Comparison of the two fixed combinations did not reveal differences in the absorption of BPO-eq between 0.1% A/BPO and 0.3% A/BPO.

The total amount of penetrated BPO-eq was not modified in regimens where BPO 2.5% was applied first nor for adapalene 0.3% for 10 min followed by the BPO 2.5% regimen. However, low BPO-eq levels were recovered when adapalene 0.1% was applied first and for adapalene 0.3% followed 10 h later by BPO 2.5%. In these regimens, the geometric mean ratio was out of the acceptance interval limits with lower BPO-eq release delivered by these regimens versus the fixed combination regimens. This could be explained by the creation of a physical barrier on the *stratum corneum* by adapalene preventing the absorption of BPO.

A limitation of these in vitro studies was the single application of the treatment regimens on the skin samples. Moreover, such studies cannot follow the bioequivalence rule of 80–125% by using 90% confidence intervals (the entire interval having to be within these limits), due to the small sample sizes. It is not possible to conclude equivalence because of variability in those studies, even if there is strict equality. Instead, these studies used the point estimate to be within 80–125% in order to conclude no difference. However, we found this to be a better rule than testing for differences by statistical tests.

Overall, the results of these in vitro studies indicated that fixed combination 0.3% A/BPO is better than 0.1% A/BPO gel, and both fixed-combination gels showed higher release of adapalene than its monads, and higher BPO release (according to treatment conditions) than its monad. The high adapalene penetration and concentration in the skin may explain the higher efficacy observed in the clinic with the fixed combination 0.3% A/BPO.

CONCLUSION

In conclusion, these in vitro studies demonstrate that fixed-combination A/BPO gels provide optimal percutaneous absorption of the active compounds compared to free-combination regimens of monad formulations of adapalene 0.1%, adapalene 0.3%, and BPO 2.5%. The higher concentration of adapalene in the 0.3% A/BPO gel and the resulting higher absorption may explain the higher efficacy in the clinic.

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Compliance with Ethics Guidelines. This article does not contain any new studies with human or animal subjects performed by any of the authors.

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