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IMPRACTICALITY EXAMINATION FOR TRANSDERMAL TRANSFER OF ELVITEGRAVIR INTERPRETATIONS

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Abstract-The aim of this study was to test the feasibility of transdermal delivery of elvitegravir (EVG), a potent HIV-1 integrase inhibitor for development of a transdermal patch of the same for HIV prophylaxis. The targeted skin permeation flux was about 25 µg/cm2/h. In vitro drug permeation studies were performed using vertical Franz diffusion cells. Passive permeation of EVG through dermatomed human skin and human epidermis was investigated. Effect of 5% w/w oleic acid, 25% v/v ethanol, 40% w/w dimethyl sulfoxide, 10% w/w lauric acid, and combination of 20% w/w dimethyl sulfoxide, 10% w/w oleic acid, and 5% w/w lauric acid in propylene glycol on the permeation of EVG through human epidermis was evaluated. Phosphate buffered saline (pH 7.4) containing 10% v/v propylene carbonate and/or polyethylene glycol 400 was used as the receptor. Sampling of the receptor was performed at pre-determined time points for 168 h and analysis was done using HPLC. Permeation of EVG through dermatomed human skin and human epidermis from its free solution in propylene glycol was found to be 5.51 ± 3.48 µg/cm2 and 21.14 ± 3.23 µg/cm2 (control), respectively after 168 h. Oleic acid and dimethyl sulfoxide significantly enhanced the permeation of EVG to 235.97 ± 49.06 µg/cm2 and 700.01 ± 107.03 µg/cm2, respectively, through human epidermis in comparison to the control group (p<0.05). Overall, even with the use of different enhancers or their combination, the targeted permeation flux was not achieved. EVG was thus, not considered further for patch development.

Keywords: Elvitegravir (EVG), Potent HIV-1, HIV prophylaxis

Introduction

EVG, a potent HIV-1 integrase inhibitor, is used in combination with other antiretrovirals, as a first line therapy for HIV infection (Y. Gong et al., 2017; Ramanathan et al., 2011). It is effective against wild as well as drug resistant HIV strains (Ramanathan et al., 2011). In addition to the therapeutic effects, EVG has also been investigated for its efficacy as part of a multiple-drug regimen (emtricitabine, cobicistat, tenofovirdisoproxilfumarate) for post-exposure prophylaxis (Mayer et al., 2017). Pharmacokinetic studies in macaques demonstrated EVG as one of the promising HIV prophylactic agents (Massud et al., 2014). Single oral dose of EVG (combination with emtricitabine, cobicistat, tenofovirdisoproxilfumarate: available as STRIBILD®) has been reported to be efficacious for HIV treatment (Desimmie, Schrijvers, &Debyser, 2012; Ramanathan et al., 2011). The absolute oral bioavailability of EVG has not been determined in humans. However in animals, it has been reported to have moderate oral bioavailability (~30%), probably due to extensive first pass metabolism (Ramanathan et al., 2011). EVG also displays non- linear pharmacokinetics that indicates its solubility-limited absorption (Deeks, 2014). Transdermal drug delivery systems have the potential to bypass the hepatic first- pass effect, resulting in improvement of EVG's bioavailability. In addition to enhancing the bioavailability, if an intermediate use transdermal dosage form (3-7 day patch) be made available for EVG for HIV prophylaxis, it would be more patient compliant. This may be attributed to the various advantages of transdermal delivery systems over the short-acting tablets and topical gels and long-acting intravaginal rings, injectables, and implants, currently being investigated for different drugs for HIV prevention (Puri, Sivaraman, et al.,

2017). The major challenge in developing transdermal drug delivery systems is to ensure permeation of drugs across stratum corneum, the outermost dead barrier layer of skin.

However, different enhancement technologies can be applied for improving the permeation kinetics of drugs across skin. These include the use of chemical penetration enhancers, microneedles,iontophoresis,laser ablation, microdermabrasion, and sonophoresis. (Banga, 2011; Puri, Sivaraman, et al., 2017).EVG is a lipophilic (log P = 4.67) drug with molecular weight: 447.8 g/mol, pKa: 6.5, melting point: 162.5 °C, and water solubility of <0.3 µg/mL. It is a dihydroquinoline- type carboxylic acid derivative and its chemical structure has been shown in Figure 22 (Puri, Sivaraman, et al., 2017; Ramanathan et al., 2011).

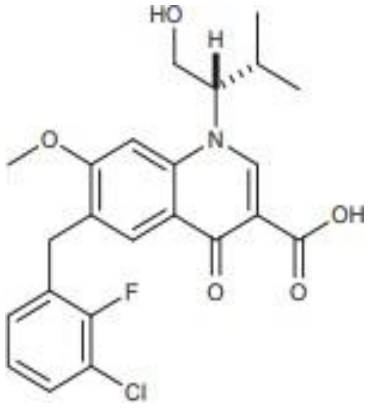

Figure 22. Chemical structure of EVG

Melting point and molecular weight of EVG seem suitable for passive permeation across skin. However, due to high log P and poor aqueous solubility, transdermal delivery of EVG was anticipated to be challenging without using any of the enhancement technologies. As most of the physical enhancement strategies (microneedles, iontophoresis, laser) are applicable and preferred for hydrophilic drugs, we employed chemical penetration enhancers, individually or in combination, for investigating transdermal permeation of EVG across human epidermis. Chemical enhancers improve drug permeation by reversible modification of the stratum corneum barrier resistance (Banga, 2011; Puri, Sivaraman, et al., 2017).

Thus, the aim of this study was to investigate the formulation feasibility of transdermal delivery of EVG by evaluating the effect of various chemical penetration enhancers on its skin permeation. Effect of oleic acid, DMSO, lauric acid, ethanol, and combination of oleic acid, DMSO, and lauric acid on permeation of EVG across human skin was evaluated in vitro.

Methods

Solubility studies

Solubility of EVG in propylene glycol; DMSO, oleic acid, lauric acid and propylene glycol (20:10:5:65); 1X PBS and PEG 400 (1:1); 1X PBS, PEG 400, and propylene carbonate (4:5:1) was determined. Composition of 1X PBS was 10 mM phosphate ions, 137 mM sodium chloride, and 2.7 mM potassium chloride and its pH was 7.4. An excess amount of drug was added to 1 mL of each solvent and was allowed to shake at room temperature at 150 rpm for 24 h. Samples were then centrifuged at 13,400 rpm for 5 min. The supernatants were diluted with methanol, filtered through 0.45 µm syringe filters (Celltreat Scientific Products, Shirley, MA, USA) and analyzed using the HPLC method described below under the section of quantitative analysis. Passive permeation of EVG. Passive permeation of EVG across both dermatomed human cadaver skin as well as epidermis, was evaluated. In this study, 500 µL of EVG solution in propylene glycol (3.24 mg/mL, equivalent to 90% saturation solubility) was added in the donor. PBS (1X): PEG 400 (1:1) containing gentamycin sulfate (80 mg/L) was used as the receptor solution. Gentamycin was added as an antibacterial as the in vitro permeation studies were performed for 7 days. The receptor (300 µL) was withdrawn at 2, 4, 6, 8, 22, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168 h and replaced with equivalent volume of fresh receptor solution. Effect of different chemical enhancers on the skin permeation of EVG. The effect of different chemical enhancers on transdermal delivery of EVG across human epidermis was investigated in this study. Drug vehicles comprised of 5% w/w oleic acid, 25% v/v ethanol, 40% w/w DMSO, and 10% w/w lauric acid, prepared individually in propylene glycol. The donor solution consisted of 500 µL of EVG in each of the vehicles. The drug concentration, sampling time points, and replacement strategy was similar to the passive permeation study.Effect of combination of enhancers on the skin permeation of EVG. The effect of combination of 10% w/w oleic acid, 5% w/w lauric acid, and 20% w/w DMSO in propylene glycol (drug vehicle) on transdermal delivery of EVG across human epidermis was investigated in this study. Donor solution comprised of EVG solution in the drug vehicle (200 µL, concentration of 31.29

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mg/mL- equivalent to 90% saturation solubility in the vehicle). PBS (1X): PEG 400: propylene carbonate (4:5:1) containing gentamycin sulfate (80 mg/L) was used as the receptor solution to maintain sink conditions. Sampling time points and replacement strategy were similar to the passive permeation study. Calculation of permeation flux and lag time. Permeation flux was calculated as the slope of linear portion of the permeation profiles (cumulative drug permeated/cm2 plotted against the time). The x-intercept of the extrapolated linear portion of the same profile indicated the lag time (Puri, Murnane, et al., 2017).

Skin extraction

After 168 h, donor formulations were removed with 3 dry cotton swabs first, followed by 2 cotton swabs soaked in receptor solution. The epidermis from each cell was then minced individually and placed in 2 mL methanol in 6 well plate. The plate was placed on a shaker (New Brunswick Scientific Co. Inc., Edison NJ, USA) for overnight shaking at 100 rpm. The samples were filtered through 0.45 µm syringe filters (Cell treat Scientific Products, Shirley, MA, USA) and analyzed using HPLC.

Data analysis

All the data was analyzed using Microsoft Excel. Unpaired Student's t-test was applied for statistical analysis and p value of less than 0.05 was considered for concluding significant difference between the test groups. All the results have been presented as mean ± SE.

Results and Discussion

Owing to the lipophilic properties of EVG (log P: 4.67 and poor aqueous solubility: <0.3 µg/mL), it was expected to have low passive skin permeation and achieving the targeted flux of 25 µg/cm2 /h was anticipated to not be possible without using different chemical and physical enhancement strategies. However, as the ultimate goal was to investigate the feasibility of formulating a transdermal patch of EVG and since most of the physical enhancement strategies are employed for hydrophilic drugs, it was considered better to investigate the effect of various chemical enhancers on its permeation across skin. Chemicals that are commonly incorporated as skin penetration enhancers in topical and transdermal products such as oleic acid, ethanol, lauric acid, and DMSO were explored for their effect on permeation of EVG.

Solubility studies

Table 3 shows the solubility of EVG determined in different solvent systems. The saturation solubility in propylene glycol as well as combination of enhancers in propylene glycol was investigated so as to be able to use 90 % saturated drug concentration as donor for the in vitro studies (providing maximum thermodynamic activity). Also, as EVG was reported to have poor aqueous solubility, solubility in mixture of 1X PBS and PEG 400/ PEG 400 + propylene carbonate was determined in order to investigate the satisfaction of sink conditions to employ these solvents as receptor solution for the in vitro permeation studies.

Table 3. Solubility of EVG in different solvents

SOLVENTS	SOLUBILITY (mg/mL)
Propylene glycol	3.6
DMSO, oleic acid, lauric acid and propylene glycol (20:10:5:65)	34.77
1X PBS and PEG 400 (1:1)	0.21
1X PBS, PEG 400, and propylene carbonate (4:5:1)	3.38

In vitro permeation studies

Passive permeation of EVG. As shown in Figure 23, permeation of EVG through dermatomed human skin and human epidermis from its solution in propylene glycol was found to be 5.51 ± 3.48 µg/cm2 and 21.14 ± 3.23 µg/cm2 (control), respectively after 168 h. In addition to 4-fold enhancement in drug permeation, lag time was reduced from 116 h to 9.5 h with the use of human epidermis as compared to dermatomed skin. These results were in agreement with those reported in the literature previously. Amalia et al. investigated the amount of nafarelin delivered across dermatomed human skin (300-500 µm in thickness) and epidermis after application of iontophoresis in an in vitro set up. The cumulative amount (nmol/cm2) of drug that was delivered after 24 h was3.97 ±

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0.1 for dermatomed full thickness skin and 28 ± 13.3 for epidermis (Rodríguez Bayón& Guy, 1996). The significant difference between drug delivery across epidermis and dermatomed skin was attributed to the kinetic effect associated with longer diffusional path length in case of the thicker skin piece (Rodríguez Bayón& Guy, 1996).Thus, the same reasoning may support the observations made in our study as well. Also, due to lesser diffusional pathlength for the drug in case of epidermis, in addition to observance of more drug in the receptor, it was detected in the receptor earlier (9.5 h) as compared to dermatomed skin (116 h).

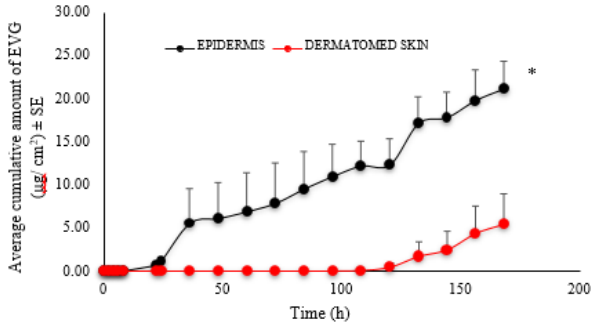

Figure 23. Passive permeation profile of EVG

In case of transdermal delivery, where the drug is expected to reach the systemic circulation, human epidermis has been stated as a better model than full-thickness and dermatomed human skin for in vitro studies. This has been explained as in actual in vivo conditions, drugs are required to successfully cross the epidermis in order to reach the blood circulation as the latter lies just below the epidermal-dermal junction. The use of full-thickness or dermatomed skin in an in vitro set up may actually underestimate the amount of drug that can be delivered in an in vivo scenario (Banga, 2011). Therefore, as EVG was intended to be delivered transdermally, human epidermis was selected for the future studies.Effect of chemical enhancers on skin permeation of EVG. The amount of EVG in receptor and skin was analyzed. Amount of EVG in receptor. Oleic acid (5% w/w) significantly enhanced the permeation of EVG to 235.97 ± 49.06 µg/cm2 through human epidermis in comparison to the control group ($p<0.05$) as shown in Figure 24. However, the lag time for drug permeation was observed to be about 19 h. Incorporation of 40% w/w DMSO into the donor formulation, further enhanced the permeation of EVG to 700.01 ± 107.03 µg/cm2 as shown in Figure 24 and reduced the lag time to 3.8 h as compared to the control group ($p<0.05$). The average permeation flux observed with passive, oleic acid, and DMSO group was 0.12 ± 0.02, 1.76 ± 0.53, and 5.3 ± 1.58 µg/cm2/h, respectively. However, targeted flux of 25 µg/cm2/h for HIV prophylaxis could not be achieved with oleic acid and DMSO. Further, ethanol (6.74 ± 1.84 µg/cm2) and lauric acid (26.53 ± 4.16 µg/cm2) did not show any enhancement in the permeation of EVG as compared to the control after 168 h, but effectively reduced the lag time to 1.03 h and 1.22 h as can be seen from the day 1 permeation profile (Figure 24, 25). EVG solution containing lauric acid was observed to gel after 24 h. The comparison of lag times observed in case of different enhancers has been depicted in Figure 26.

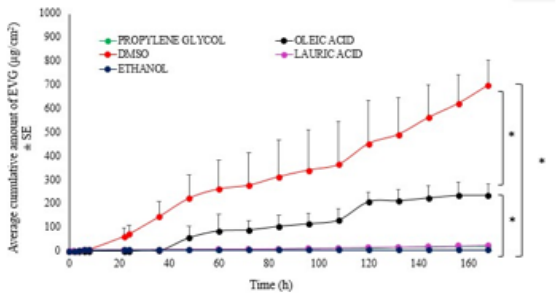

Figure 24. Effect of chemical enhancers on EVG permeation through human epidermis
* denotes significant difference ($p<0.05$) as compared to other groups, Student's t test

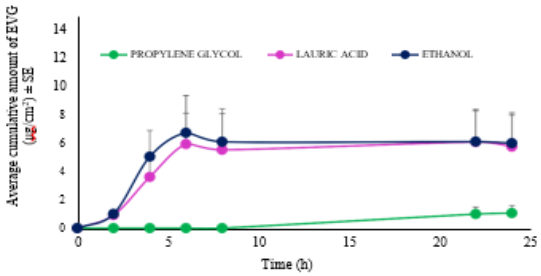

Figure 25. Effect of ethanol and lauric acid on EVG permeation through human epidermis during

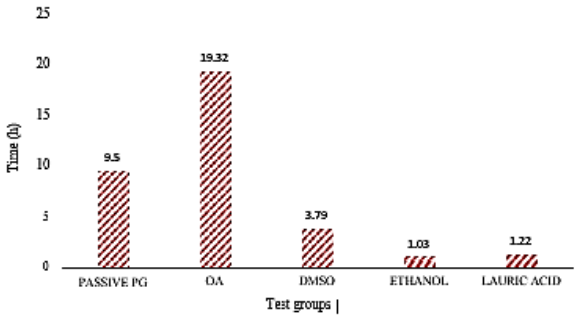

Figure 26. Lag times for EVG permeation through human epidermis in different test groups

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DMSO has also been reported to enhance the transdermal permeation of drugs. Mechanistically, it acts by inducing conformational changes in keratin, interacting with the alkyl chains of stratum corneum lipids, displacing water bound with keratin, and extracting lipids from skin (Lane, 2013). Thus, overall due to these effects on the barrier layer of skin, DMSO probably facilitated increased delivery of EVG across the epidermis layer.Ethanol and lauric acid are also commonly employed skin permeation enhancers. However, they were not able to significantly enhance the delivery of EVG across human epidermis. But, they considerably reduced the lag time to about 1 h as compared to 9.5 h in passive and 19 h in oleic acid group. Therefore, EVG permeated faster in presence of ethanol and lauric acid as compared to other test groups. In the former, this can be explained due to the evaporative loss of ethanol from the formulation that results in increase in the drug concentration beyond saturation, providing a supersaturated state with a greater driving force for permeation. Furthermore, ethanol itself rapidly permeates into skin (steady state flux ~ 1 mg/cm2/h) and thus, can carry the permeants along with it (Berner et al., 1989). Therefore, higher amount of drug permeation was observed in presence of ethanol than the passive group only in the initial hours of permeation. As the permeation study was performed for 7 days and ethanol would have evaporated in the initial hours itself, no overall enhancement in drug delivery was observed. Lauric acid has previously been investigated as a transdermal permeation enhancer, even though its mechanism of action is not clearly understood. Lauric acid in propylene glycol (10% w/w) has previously been demonstrated to work as an effective combination for enhancing the delivery of highly lipophilic drugs across mice skin (Funke et al., 2002). However, in our study, solution of EVG in lauric acid-propylene glycol mixture was found to gel after day 1 and some crystals were also visible in the formulation. This may have happened due to incompatibility between EVG and lauric acid. However, during day 1, before gelling and crystallization was evident, EVG was found to permeate across skin at a faster rate as compared to passive and oleic acid group. This was understood from the lag time of 1 h observed for lauric acid group.

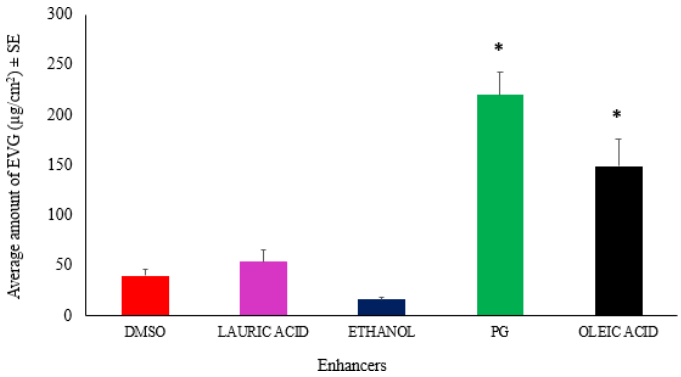

Figure 27. Amount of EVG retained in human epidermis after 7 days

* denotes significant difference ($p<0.05$) as compared to other groups, Student's t testEVG amount in skin.

As shown in Figure 27, oleic acid (148.66 ± 26.91 µg/cm2) and passive group (219.56 ± 23.46 µg/cm2) showed highest drug retention in epidermis than other groups ($p<0.05$). In case of passive group, propylene glycol alone facilitated penetration of EVG across stratum corneum, but most of the drug amount was retained in the epidermis and did not reach the receptor. However, oleic acid was effective in enhancing permeation of EVG into as well as across epidermis. This may be attributed to the permeating enhancing effect of oleic acid as well as its lipophilic nature that may have resulted in its binding with skin and thus, drug retention as well. However, DMSO did not facilitate in retaining high EVG amount in the skin (40.00 ± 6.18 µg/cm2), but significantly enhanced drug permeation across epidermis (700.01 ± 107.03 µg/cm2). With ethanol and lauric acid, significantly low amount of EVG was observed in the epidermis (15.97 ± 2.67 µg/cm2 and 53.60 ± 11.52 µg/cm2, respectively) as compared to propylene glycol and oleic acid groups ($p<0.05$). Thus, in these two cases, most of the drug remained unabsorbed (in the donor itself). In case of passive as well as ethanol and lauric acid, more drug was obtained in the skin than receptor. This may be attributed to the lipophilic properties of EVG (log P: 4.67) that may have resulted in its binding to lipids in stratum corneum and thus, more retention than permeation across epidermis. Effect of combination of chemical enhancers on skin permeation of EVG. Combination of 20% w/w DMSO, 10% w/w oleic acid, and 5% w/w lauric acid in propylene glycol was selected based on the observations with the studies conducted with individual enhancers. As DMSO and oleic acid were found to be effective in the abovementioned studies, they were selected

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for the combination study, but as 40% DMSO was too high from the perspective of including in a transdermal patch, therefore we used 20% w/w. However, oleic acid was enhanced from 5 to 10% w/w to explore the effect of increasing its concentration. Also, as lauric acid was successful in reducing the lag time for drug permeation, it was included in the combination study. However, due to crystallization issues with 10% w/w lauric acid, its concentration was reduced to 5% w/w in this study. Also, as our aim was to assess the feasibility of achieving transdermal permeation flux of EVG of 25 µg/cm2/h, drug concentration in the donor was increased and corresponded to the saturation solubility of EVG in the combination of enhancers.

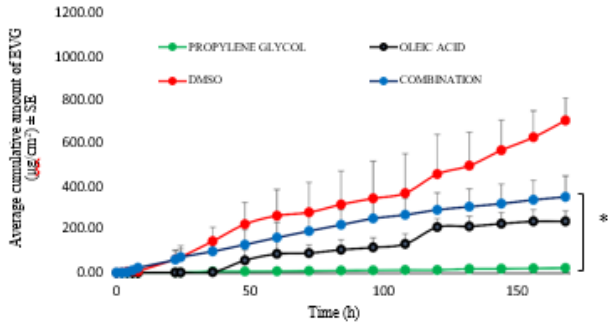

Figure 28. Effect of combination of enhancers on EVG permeation through human epidermis

* denotes significant difference ($p<0.05$) as compared to other groups, Student's t test

As depicted in Figure 28, combination of 20% w/w DMSO, 10% w/w oleic acid, and 5% w/w lauric acid in propylene glycol delivered 348.71 ± 96.50 µg/cm2 of EVG in the receptor after 168 h, which was significantly higher than the control group ($p<0.05$) but not as compared to DMSO and oleic acid group ($p>0.05$). Permeation flux of 2.18 ± 0.49 µg/cm2 and lag time of 1.08 h was observed with the combination of penetration enhancers. Also, the formulation was observed to gel after 24 h, that slowed down the permeation of EVG and thus, significant enhancement in delivery of EVG (that was expected) was not observed.Thus, overall, none of the enhancers evaluated provided the targeted permeation flux for EVG, even after being added at the maximum possible concentrations. Maximum average permeation flux of about 5.3 ± 1.58 µg/cm2/h was achieved using DMSO, but that was also 5 fold lesser than the target. Therefore, due to high dose requirements of EVG for HIV prophylaxis and its poor permeation profile across human epidermis in the solution studies, it was not considered as a good candidate for patch formulation and further studies were not performed.

Conclusion

EVG was observed to permeate passively through human dermatomed skin as well as epidermis. However, the drug permeation flux without using chemical enhancers was low and showed high lag time for permeation. Oleic acid (5% w/w) and DMSO (40% w/w) were found to be most effective in significantly enhancing the amount of EVG permeation through human epidermis. Ethanol and lauric acid did not improve the transdermal permeation of EVG, but shortened the lag time considerably as compared to the control and other enhancers. However, as the targeted permeation flux for HIV prophylaxis could not be achieved for EVG with the use of different enhancers, it was not considered as a suitable drug candidate for transdermal patch formulation.

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