Then, to exert the therapeutic effect, amino acid moieties of ACV pro-drugs must be cleaved by skin esterases, exposing the ACV terminal hydroxyl group for phosphorylation by viral thymidine kinase and human phosphorylase, as ACV triphosphate is the active form that inhibits viral DNA replication (Gnann et al. 1983). Indeed, synthesized pro-drugs ACV-Trp, ACV-Phe, ACV-Ile, ACV-Val, ACV-Gly, and ACV-Arg had solubility enhancements of 6, 33, 38, 48, 56, and >90-fold (by molar concentration), respectively, as compared to ACV, which could be attributed to the effect of hydrogen-bonded solvation of the ionizable group(s). Such solubility increments facilitated their formulation, as they were prepared in aqueous solution at a patient-friendly pH 5.5. As expected, the passive diffusion of ACV and ACV-X pro-drugs into and across porcine skin from 5 mM aqueous solutions was negligible after two hours; neither skin retention nor cumulative permeation was quantifiable. Iontophoresis resulted in therapeutically relevant drug amounts in the skin. The highest skin deposition of either the pro-drug or the ACV already biotransformed were achieved with the di-protonated pro-drug, ACV-Arg (785.9 ± 78.1 nmol/cm^2), which had the highest charge–mass ratio, highest solubility, and lowest log D (5.24×10^3, >820 mM, −6.08, respectively) among the six pro-drugs; therefore, its electromigration was presumably the highest under a given electric potential gradient. Iontophoresis of the two aromatic amino acid ester pro-drugs, ACV-Trp and ACV-Phe, led to the lowest skin deposition of ACV species (156.1 ± 76.3 and 249.8 ± 81.4 nmol/cm^2, respectively). Conversely to ACV-Arg, they had the lowest charge–mass ratios and solubilities and higher log D (2.43×10^3, 51.3 mM, −0.73, and 2.69×10^3, 304.4 mM, −1.22, for ACV-Trp and ACV-Phe, respectively) and thus presumably had lower electric mobility (Chen et al. 2016a).

Another notable aspect of the pro-drug strategy is that pro-drugs for iontophoresis may be designed for either topical or transdermal delivery based on the stability of the linkage to the charged moiety. Comparatively stable pro-drugs might have a higher probability of reaching the systemic circulation during iontophoresis. In contrast, enzymatically labile pro-drugs would be more likely to undergo hydrolysis earlier during cutaneous transit and release an uncharged parent drug that would be retained within the membrane. This was observed with the acyclovir pro-drugs, as total delivery of ACV-Arg, the most biolabile pro-drug, was dominated by ACV deposition, while a higher absolute amount and percentage of the applied ACV-Ile, the most enzymatically stable pro-drug, permeated across the skin as a function of time (Chen et al. 2016a). Further biodistribution studies demonstrated that while passive delivery of ACV or penciclovir from marketed cream and ointment formulations after application for 60 minutes resulted in modest cutaneous deposition, mainly in the stratum corneum or superficial viable epidermis, iontophoresis of ACV-Ile or ACV-Arg for only 10 minutes at 0.25 mA/cm^2 resulted in, respectively, twelvefold and twentyfold higher deposition of ACV species, but at deeper skin layers (100 to 200 μm) (Figure 46.3), corresponding to the basal epidermis and adjacent area, the target region for delivery where the virus would be found (Chen et al. 2016b).

The concept that pro-drug enzymatic stability leads to deeper drug penetration may even apply to biological barriers other than the skin. The ocular iontophoretic delivery of ACV-Gly, one of the ACV pro-drugs with the highest relative enzymatic stability in ocular tissues (half-life from 1.58 ± 0.13 to 5.54 ± 0.82 hours in the choroid/retina and vitreous humor extracts, respectively), showed considerable delivery of ACV species to the choroid/retina and vitreous humor (5.7 ± 2.3 and 11.7 ± 3.7 nmol/cm^2, respectively) after only five minutes transscleral iontophoresis to intact porcine eye globes (Chen and Kalia 2018). Nonetheless, other less intuitive aspects may also need to be considered such as the influence of polar surface area, dipole moment, charge, and possible tissue interactions, e.g. melanin binding in ocular delivery (Santer et al. 2018). This was observed after short-duration transscleral iontophoretic delivery of four triamcinolone acetonide (TA) amino acid ester prodrugs (TA-AA) (alanine, Ala; arginine, Arg; isoleucine, Ile and lysine, Lys) using whole porcine eye globes *in vitro*. Results showed TA-Ala had a similar ocular biodistribution profile to TA-Lys, even though TA-Lys was more enzymatically stable in the ocular tissues than TA-Ala and had a net difference in charge, being dicationic, while TA-Ala was monocationic. Such results were attributed to the presence of localized charge centers on the molecular surface, which increased