Review Article

Synergistic Effect of Enhancers for Transdermal Drug Delivery

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Transdermal drug delivery offers a non-invasive route of drug administration, although its applications are limited by low skin permeability. Various enhancers including iontophoresis, chemicals, ultrasound, and electroporation have been shown to enhance transdermal drug transport. Although all these methods have been individually shown to enhance transdermal drug transport, their combinations have often been found to enhance transdermal transport more effectively than each of them alone. This paper summarizes literature studies on these combinations with respect to their efficacy and mechanisms.

INTRODUCTION

Transdermal drug delivery offers several advantages over traditional drug delivery systems such as oral delivery and injection including elimination of first pass metabolism, minimization of pain, and possible sustained release of drugs (1). However, transdermal transport of molecules is slow due to low permeability of stratum corneum, the uppermost layer of the skin. Various physico-chemical penetration enhancers including ultrasound (2–8), chemical enhancers (9,10) iontophoresis (11), and electroporation (12) have been used for enhancing transdermal drug transport. These enhancers increase transdermal transport through one or more of the following mechanisms: i) increased drug solubility (chemical enhancers), ii) increased diffusion coefficients (chemical enhancers, ultrasound, and electroporation), and iii) provision of additional driving forces (ultrasound, iontophoresis, and electroporation).

While all these enhancers have been individually shown to enhance transdermal drug transport, their combinations have been hypothesized to be more effective compared to each of them alone. Over the last 10 years several papers have been published to support this hypothesis. Specifically, the following combinations have been used for transdermal drug delivery: i) Chemicals + Iontophoresis (13–24, 42–46), ii) Chemicals + Electroporation (25–28,51), iii) Chemicals + Ultrasound (9,29,30), iv) Iontophoresis + Ultrasound (31), v) Electroporation + Iontophoresis (32,33), and finally v) Electroporation + Ultrasound (34), see also Fig. 1. In addition to increasing transdermal transport, a combination of enhancers should also reduce the severity of the enhancers required to achieve the desirable drug flux. Specifically, the enhancement induced by these enhancers depends on their strength. However, the highest strength of the enhancers that can be applied on the skin is typically limited by safety. By combining two or more enhancers, one can reduce the strength of individual enhancers required to achieve the desired enhancement. Hence, a combination of two or more enhancer may not only increase the total enhancement, but can also increase the safety of enhancers. A review literature describing synergistic combinations of various enhancers is presented in this paper.

SPECIFIC EXAMPLES

Iontophoresis and Chemical Enhancers

Iontophoresis enhances transdermal drug transport via direct electrophoresis, electroosmosis, or enhanced diffusion (11,35,36). On the other hand, chemical enhancers increase transdermal drug transport via several different mechanisms, including increased drug solubility, increased drug partitioning into the SC, fluidization of lipid bilayers, and disruption of the intracellular proteins (29,37–40). Detailed reviews on iontophoresis and chemical enhancers may be respectively found in Refs. (11,35,36) and (39,41). Iontophoresis was one of the first enhancers to be combined with other enhancers. Srinivasan et al. showed that skin pretreatment with ethanol enhanced the effect of iontophoresis on transdermal leuprolide delivery by several-fold (13). Several reports followed this study that also documented the synergistic effect between chemical enhancers and iontophoresis. Choi et al. showed that application of chemical enhancers and iontophoresis increased transdermal insulin flux significantly more than that induced by iontophoresis alone (21). The synergistic effect between these methods was attributed to "increasing intercellular spacing due to chemical enhancers". Specifically, chemical enhancers dilated intercellular spaces and reduced

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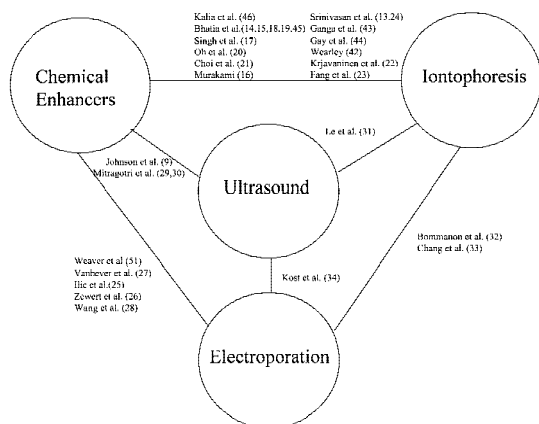
Fig. 1. The Figure shows various combinations of enhancers that have been studied. The circles indicate four major enhancers that are used for enhancing transdermal transport, that is, chemical enhancers, ultrasound, iontophoresis, and electroporation. The lines joining various circles correspond to various combinations that have been reported in the literature. The names of the investigators who performed these studies are also shown.
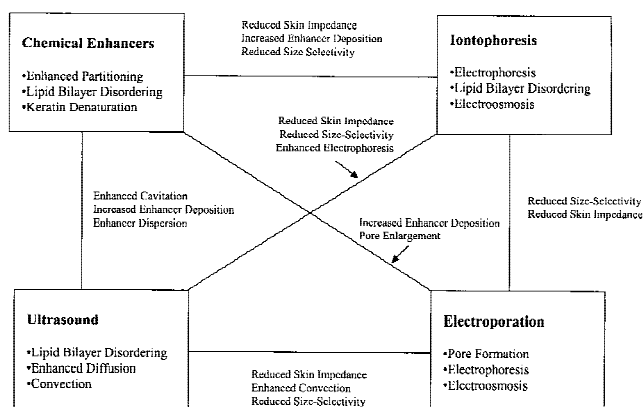
skin impedance, thus increasing the effectiveness of iontophoresis.

Oh et al. showed that propylene glycol and oleic acid enhanced transdermal transport of AZT synergistically in combination with iontophoresis (20). Application of propylene glycol and oleic acid enhanced transdermal flux of AZT by about 200-fold. On the other hand, application of iontophoresis alone enhanced AZT flux by 7-fold. However, the combination of propylene glycol and iontophoresis enhanced AZT flux by about 400-fold. The effect of these enhancers on transdermal transport was shown to be synergistic, that is, the effect of the combination was higher than the additive effect of each enhancer. On the other hand, Wearley et al. studied the effect of DMSO with iontophoresis and found that this combination did not enhance transdermal transport better than that induced by iontophoresis alone (42). Ganga et al. investigated the effect of Azone on iontophoretic transport of metoprolol (43). They found that the combination of Azone

Fig. 2. The Figure shows possible mechanisms for the synergistic effects between various enhancers. Four enhancers, including chemical enhancers, ultrasound, iontophoresis, and electroporation are listed in each box. Mechanisms responsible for each enhancer are also listed. Possible mechanisms responsible for the synergistic effect of these enhancers are listed on the lines joining respective boxes.

and iontophoresis enhanced transdermal drug transport synergistically. Similar conclusions were reached by Gay et al. who showed that oleic acid treatment enhanced iontophoretic transport of piroxicam (44). Finally, Bhatia et al. showed that skin pretreatment with chemicals increased the effect of iontophoresis on transdermal transport of LHRH (45). Specifically, application of iontophoresis alone enhanced LHRH flux by about 4-fold. On the other hand, application of 5% limonene enhanced LHRH flux by about 3-fold. However, a combination of iontophoresis and 5% limonene enhanced LHRH flux by more than 10-fold. Similar results were reported by the same investigators for other drugs including cholecystokinin-8 (14,18,19).

The synergistic effect between chemicals and iontophoresis may be attributed to several mechanisms, see Fig. 2. First, if the enhancer is charged, iontophoresis should increase the rate of enhancer delivery into the skin. This should further increase transdermal drug transport. Synergistic effect between iontophoresis and sodium lauryl sulfate may be an example of such effect (46). Specifically, Kalia et al. showed that addition of 0.25% sodium lauryl sulfate dramatically amplified the effect of iontophoresis on skin impedance in human volunteers (46). Application of SLS alone did not affect skin impedance. On the other hand, application of intophoresis alone decreased skin impedance (at 10 Hz) to about 20 kOhm. However, application of 0.25% SLS with iontophoresis decreased skin impedance (at 10 Hz) to about 5 kOhm. Similar results were obtained with other enhancers including ethanol, oleic acid, linoleic acid, and Azone. Second, penetration of enhancers into the skin may affect the lipid bilayers of the skin, thus reducing skin's electrical impedance and size-selectivity. This, in turn, should increase the transport number of larger solutes, thereby increasing the rates of drug delivery. Indeed, Bhatia et al. suggested that penetration of chemicals such as limonene into skin induced structural alterations in the lipids and proteins of the SC that created permeability defects. These defects were then utilized by iontophoresis, thus enhancing the effect of iontophoresis on transdermal drug transport.

The major advantages of iontophoresis + chemicals over each of them alone include its effectiveness and ease of application. Specifically, the device (and the process) required to implement the combination of iontophoresis with chemical enhancers is not likely to be significantly more complicated compared to that required for iontophoresis alone. In addition, a combination of a bilayer disruptor (chemical enhancer) and a driving force provider (iontophoresis) is natural. Potential issues in this method include the increased delivery of chemical enhancers into the skin, which may escalate safety concerns. In addition, iontophoresis may also deliver enhancers deeper into the skin compared to passive diffusion, thus potentially exposing subcutaneous tissues to a higher concentration of chemical enhancers. These issues should be further investigated.

Ultrasound and Chemical Enhancers

Ultrasound enhances transdermal transport via enhanced diffusion (through disordering of lipid bilayers) or enhanced convection. A detailed discussion of the effect of

ultrasound on transdermal transport can be found in Refs. (2,4,47). Three literature reports have confirmed the synergistic effect between ultrasound and chemical enhancers (9, 29, 30). Johnson et al. performed a study of the synergistic effect of therapeutic ultrasound (1 MHz, 2 W/cm^2) with a series of chemical enhancer formulations, including (i) polyethylene glycol 200 dilaurate (PEG), (ii) isopropyl myristate (IM), (iii) glycerol trioleate (GT), (iv) ethanol/pH 7.4 phosphate buffered saline in a one-to-one ratio (50% EtOH), (v) 50% EtOH saturated with linoleic acid (LA/EtOH), and (vi) phosphate buffered saline (PBS), using corticosterone as a model drug (9). A combination of LA/EtOH and ultrasound was most effective in enhancing transdermal drug transport. The combination of LA/EtOH with ultrasound increased corticosterone flux by up to 13,000-fold, relative to the passive flux from PBS. This enhancement was substantially higher than that induced by LA/EtOH alone (900-fold) or ultrasound alone (5-fold).

Johnson et al. suggested that the primary mechanism of the synergistic effect of ultrasound and chemicals is the mixing of the chemical enhancer with SC lipids upon ultrasound application. Specifically, studies have shown that fatty acids, such as oleic acid, form segregated phases within the SC (48). Under passive conditions, linoleic acid may also tend to diffuse into the SC and collect in pools. Ultrasound may induce mixing and facilitate the dispersion of linoleic acid and the SC lipids. The increased entropy of the resulting mixed system would make it a more favorable molecular arrangement, which would remain stable even after ultrasound is turned off.

Recently, Mitragotri et al. (29,30) performed an evaluation of the synergistic effect of low-frequency ultrasound (20 kHz) with sodium lauryl sulfate (SLS) and a model permeant, mannitol. Application of SLS alone as well of ultrasound alone increased skin permeability. Application of SLS alone for 90 minutes induced about 3-fold increase in mannitol permeability, while application of ultrasound alone for 90 minutes induced about 8-fold enhancement. However, when combined, application of ultrasound from 1% SLS solution induced about 200-fold increase in skin permeability to mannitol. Ultrasound also reduced the threshold ultrasound energy required to induce a detectable change in skin permeability. Specifically, in the absence of surfactants, the threshold ultrasound energy for producing a detectable change in skin impedance is about 141 J/cm^2. Addition of 1% SLS to the solution decreased the threshold to about 18 J/cm^2 (29). Various possible mechanisms of this synergistic effect were investigated. These include: i) SLS enhances ultrasound-induced cavitation, ii) Ultrasound drives more SLS into the skin, and iii) Ultrasound may enhance dispersion of SLS within the SC lipids. The latter two mechanisms were found to be dominant.

Combination of ultrasound and chemical enhancers offers several advantages over the use of ultrasound or chemicals alone including its high efficiency and ease of application. As in the case of iontophoresis + chemical enhancers, the required device and the process for application of ultrasound + chemicals is not significantly more complex compared to that required for ultrasound alone. Note that although both ultrasound and chemical enhancers are bilayer disruptors, their combination exhibits significant synergistic effect. Potential limitations of this combination may include increased concentration and deep penetration of chemicals in the skin.

Ultrasound and Iontophoresis

Synergy between ultrasound and iontophoresis is expected since these enhancers increase transdermal transport through different mechanisms. Indeed, this combination has been found to enhance transdermal transport better than each of them alone. Specifically, Le et al. performed an investigation of the synergistic effect of ultrasound and iontophoresis on transdermal transport using a model drug, heparin (31). Ultrasound was applied only once to each skin piece (along with 1% solution of dodecyl pyridinium chloride) for about 10 minutes prior to application of iontophoresis. The enhancement of heparin flux due to ultrasound+iontophoresis treatment was about 56-fold (note that iontophoresis was applied only for 1 hour). This enhancement was higher than the sum of those obtained during ultrasound alone (3-fold) and iontophoresis alone (15-fold). Thus, the effect of ultrasound and iontophoresis on transdermal heparin transport is truly synergistic.

The synergistic effect of ultrasound and iontophoresis on transdermal transport was attributed to ultrasound-induced structural changes in the skin. Specifically, application of ultrasound should disorder the lipid bilayers of the skin, thereby introducing new transport pathways. The presence of these pathways decreases skin's impedance and size-selectivity. Both these effects should result in increased transdermal transport. The authors suggested that a combination of ultrasound and iontophoresis offers significant benefits over either of them alone including enhancement of transdermal flux, and reduction of the required voltage/current to achieve the desired flux. Further studies are required to investigate the mechanisms of this synergy. The advantages of this combination include the fact that ultrasound and iontophoresis enhance transdermal transport through different mechanisms, thus making this combination very natural. The limitations of this method may include the possibility of requiring a relatively complex device compared to ultrasound or iontophoresis alone.

Ultrasound and Electroporation

Electroporation enhances transdermal transport through enhanced diffusion (via skin poration), electrophoresis, and electroosmosis. A detailed discussion on the mechanisms of electroporation can be found in Refs. (12,49,50). Kost et al. investigated the synergistic effect of therapeutic ultrasound and electroporation on transdermal transport of two molecules, calcein and sulforhodamine (34). Application of ultrasound (1 MHz, 2 W/cm^2) did not enhance transdermal calcein flux, while application of electroporation alone enhanced transdermal calcein transport to 0.1 μg/cm^2/hr. However, a simultaneous application of ultrasound and electroporation enhanced transdermal calcein transport to 0.3 μg/cm^2/hr. Application of ultrasound also reduced the threshold voltage for electroporation. The threshold voltage for electroporation (under the protocol described in Ref. (34)) is about 53 ± 3 V in the absence of ultrasound and about 46 ± 3 V in the presence of ultrasound. The voltage required to achieve a given transdermal flux was also smaller in the presence of ultrasound. For example, to achieve a transdermal sulforhodamine flux of 0.15 μg/cm^2/hr, the required voltage is about 95 V in the absence of ultrasound and 75 V in the presence of ultrasound.

The authors suggested that ultrasound might play a two-fold role in enhancing the effect of electroporation on transdermal transport. First, ultrasound may induce partial structural disordering of the skin's lipid bilayers. Since the electrical resistance of the disordered bilayers is likely to be smaller than that of the normal lipid bilayers, the applied voltage may concentrate preferentially across the normal bilayers. Second, ultrasound may also induce convection across the skin. Both effects were found to play important roles in the synergistic effect of ultrasound and electroporation on transdermal transport of calcein and sulforhodamine. Detailed studies are required to understand the mechanisms of this synergy further.

This method is likely to provide similar advantages compared to that offered by iontophoresis and ultrasound. At the same time, this method suffers from the limitation that both ultrasound and electroporation are bilayer disrupting agents, thus making their combination somewhat unnatural. The device requirement for this combination is also expected to be more complex compared to that for ultrasound or electroporation alone.

Iontophoresis and Electroporation

Although extensive studies can be found on the use of iontophoresis or electroporation alone on transdermal transport, only a few studies explicitly focused on the synergistic effect of iontophoresis and electroporation. Bommanon et al. studied the synergistic effect of iontophoresis and electroporation on transdermal delivery of LHRH in vitro (32). Fluxes achieved with and without electroporation under different iontophoretic current densities (0– 4 mA/cm^2) were compared. The results indicated that application of a single electroporation pulse prior to iontophoresis consistently yielded 5-10 fold higher fluxes. The increased efficiency of electroporation + iontophoresis was attributed to the reduced impedance and size-selectivity of the skin. Recently, Chang et al. studied the effect of iontophoresis and electroporation on transdermal transport of salmon calcitonin and parathyroid hormone through human epidermis (33). The authors reported that a combination of electroporation and iontophoresis induced higher transdermal permeation than that induced by either one technique alone. Specifically, transdermal calcitonin fluxes due to electroporation alone or iontophoresis alone were respectively <20 ng/cm^2/hr and about 200 ng/cm^2/hr. However, application of electroporation prior to iontophoresis increased calcitonin flux to about 800 ng/cm^2/hr. Electroporation also shortened the lag time of iontophoretic transdermal delivery of salmon calcitonin.

The mechanism for the synergistic effect of electroporation and iontophoresis is likely to be analogous to that of ultrasound and iontophoresis. Specifically, electroporation may create new transport pathways in the SC, thus facilitating passage of current during iontophoresis. The advantages of this combination include the difference between the mechanisms of action of these enhancers, thus making the combination natural. The device requirements are also likely to be comparable to those for electroporation alone.

Electroporation and Chemical Enhancers

Several literature reports indicate the synergistic effect between electroporation and chemicals (26,27,51). These chemicals include polysaccharides (heparin and dextran), urea, and sodium thiosulfate. Note that these chemicals are very different than those discussed commonly in the chemical enhancer literature (for example, fatty acids and surfactants). Vanbever et al. showed that the combination of electroporation and polysaccharides is more effective than electroporation alone in enhancing transdermal transport (27). Specifically, electroporation increased transdermal mannitol delivery by approximately two orders of magnitude. The addition of macromolecules further increased mannitol transport by up to five-fold. Although all macromolecules that they studied enhanced transport, those with greater charge and size were more effective. The authors claimed that because heparin molecules are long enough to span several lipid bilayer membranes that separate keratinocytes within the SC, these results could be explained by the hypothesis that heparin molecules were trapped within the skin, holding open pathway segments connecting adjacent keratinocytes.

Recently, Zewert et al. hypothesized that a combination of electrical field and chemicals (topical sodium thiosulfate) can be used to create enlarged aqueous pathways that allow large quantities of macromolecules to be transported through the SC (26). In vitro experiments on human skin demonstrated that this combination enhances transdermal transport of proteins by several orders of magnitude. In the absence of sodium thiosulfate, electroporation enhanced transdermal flux of only small molecules (for example, sulforhodamine). Significant macromolecular fluxes occurred only if a pathway-enlarging molecule (sodium thiosulfate) was present. In a later study, the authors showed that SC-spanning microconduits (diameters of about 200 micron) could be created in vivo using the same combination (25). A single microconduit in isolated SC supported a volumetric flow of the order of 10 μl/s ml by a pressure difference of only 0.01 atm. Authors hypothesized that the synergistic effect of thiosulfate and electroporation on transdermal transport owes to the fact that while electroporation opens up new pathways in lipid bilayers, thiosulfate disrupts disulfide bonds in keratin. Thus, this combination of enhancers opens up pathways that were not otherwise available for transport.

The combination of electroporation and chemicals offers advantages over electroporation alone in that the device requirements are not significantly different than those for electroporation alone. Potential limitations of this technique may include increase concentration and deeper penetration of chemicals in the skin.

CONCLUSION

Various enhancers including chemicals, electric fields, and ultrasound have been used to enhance transdermal drug transport. Although all these enhancers have been individually shown to enhance transdermal drug transport, their combinations are significantly more effective compared to each of them alone. In most cases, the enhancement is synergistic, that is, the enhancement induced by the combination of enhancers is higher than the sum of the enhancement induced by each enhancer alone. Such combinations offer an advantageous method of transdermal drug delivery. Additional research on such combinations with respect to their safety and efficacy should be performed to further assess their complete potential.

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