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# Investigation into the potential of low-frequency ultrasound facilitated topical delivery of Cyclosporin A

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**Abstract**

The potential for low-frequency ultrasound facilitated topical transport of Cyclosporin A was investigated using rat skin. Studies of intensity and exposure time acting on the deposition of Cyclosporin A into deeper skin of in vitro sonophoresis were performed. Low-frequency ultrasound increased the amount of Cyclosporin A retained in the skin only seven times than the passive diffusion. Furthermore, we also tested the synergistic effect of ultrasound and other approaches such as chemical enhancers and electroporation on topical drug delivery of Cyclosporin A. We found that the efficacy of low-frequency ultrasound in enhancing topical delivery could be further increased by pretreatment of skin with chemical enhancers, such as laurocapram (Azone) and sodium lauryl sulfate (SLS). Meanwhile only a small amount was seen to across the full skin into the receiver compartment. Trimodality treatment comprising of pretreatment with Azone + ultrasound in combination followed by electroporation was not effective in enhancing the topical delivery of Cyclosporin A. However, this combination strategy increased the penetration of Cyclosporin A through rat skin by order of 15. The histopathological findings revealed that there was almost no change observed in the structure of skin after ultrasound or combination with ultrasound and enhancers as compared with the control group. In general, the enhanced skin accumulation of Cyclosporin A by the combination of low-frequency ultrasound and chemical enhancers could help significantly to optimize the targeting of the drug without of a concomitant increase of the systemic side effects.


*Keywords:* Low-frequency ultrasound; Chemical enhancer; Eletroporation; Synergistic; Cyclosporin A

## 1. Introduction

Cyclosporin A (CysA, $M_W$ 1203 Da) is a nonpolar cyclic oligopeptide consisting of 11 amino acids (Lallemand et al., 2003). Over the past years, CysA has been evaluated for numeruous potential application in dermatology. It is effective in the treatment of alopecia areata and psoriasis, when administered systemically by i.v. injection or oral application (Hultsch et al., 2005; Vc, 2004). However, long-term systemic administration of CysA has been noted to produce harmful effects such as hypochromic, granulomatous, hypatitis and proximal renal tubular cell damage (Guzzo, 1997; Zachariae et al., 1997). This drawback has led to the exploration of investigations using CysA to achieve local immune suppression. Topical delivery of CysA is hindered by its physicochemical properties and the barrier property of stratum corneum (SC) (Duncan et al., 1990; Choi and Flynn, 1995). Many studies have used physical and chemical techniques to disrupt the stratum corneum barrier (Verma et al., 2004; Liu et al., 2006a; Tran et al., 1999; Verma and Fahr, 2004; Boinpally et al., 2004; Wang et al., 1998). Recently, Intradermal drug delivery of CysA was reported effective in alopecia areata, melasma, and solar lentigo by low-frequency ultrasound (25 kHz) (Santoianni et al., 2004).

Low-frequency ultrasound, which is also known as sonophoresis, is the movement of drug molecules through the skin under the influence of ultrasound. Sonophoresis at various frequencies in range of 20 kHz to 16 MHz has been used to enhance skin permeability (Benson et al., 1988; Mitragotri et al., 1995, 1996). However, transdermal transport enhancement induced by low-frequency ultrasound ($f < 100$ kHz) has been found to be more significant than that induced by high frequency ultrasound (Tezel et al., 2001; Boucaud et al., 2002). Several research groups have successfully performed transdermal delivery of macromolecules with in vitro and in vivo sonophoresis

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(Tachibana, 1992; Mitragotri and Kost, 2001). The safety issues of the technique have also been addressed by Boucaud et al. (2001). Several literature reports have confirmed the synergistic effect between ultrasound and chemical or physical enhancers (Johnson et al., 1996; Mitragotri, 2000; Mitragotri et al., 1999; Kost et al., 1996). In addition to increasing transdermal transport, a combination of enhancers should also reduce the severity of the enhancers required to achieve the desirable drug delivery. 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.

The aim of the present study was to investigate the influence of intensity, exposure time and combination with other enhancement methods, such as chemical enhancers and electroporation on the topical delivery of CysA during sonophoresis.

## 2. Materials and methods

### 2.1. Materials

Cyclosporin A was procured from Sigma Chemical. Azone (laurocapram), NMP (*N*-methyl pyrrolidine), DMSO (dimethyl sulfoxide), SLS (sodium lauryl sulfate) and menthol were obtained from Shanghai Chemical Reagent Corporation (Shanghai, China). All other chemicals were of either reagent or high-performance liquid chromatography (HPLC) grade.

### 2.2. Skin preparation

All experiments were conducted according to the protocol approved by the Institutional Animal Ethics Committee (IAEC) of NIPER. Sprague–Dawley rats (200–250 g) were sacrificed by excessive ether anesthesia. Hair was removed from the abdominal portion using an animal hair clipper and full thickness skin was harvested. Then the fat adhering on the dermis side was removed by using scalpel and isopropyl alcohol. Finally, skin was washed in tap water, placed in a refrigerator at 4 °C overnight, and then used for the experiments.

### 2.3. In vitro permeation procedures

The permeation experiments were performed using modified Franz diffusion cells with diffusion area of 2.50 $cm^2$ and a receiver volume of 13.0 ml. Normal saline containing 20% ethanol was used as the receiver medium. The receptor chambers were thermostated at 32 °C and the solution in the receptor chambers was stirred continuously at 300 rpm. A suspension of 0.5% (w/w) CysA was made with normal saline containing 40% ethanol by agitating on an environment shaker at 32 ± 1 °C for 24 h. The formulations (1.0 ml) containing 0.5% (w/w) CysA were gently placed in donor chamber. All of receptor medium was withdrawn at 3 h-interval and immediately replaced by an equal volume of fresh receptor solution. The samples were assayed by HPLC.

The amount of CysA accumulated into the skin was recovered using the following procedure. To eliminate any contamination from traces of the vehicles the skin was striped with 20 pieces of adhesive tape. The stripped skin was cut into small pieces and vortex-mixed for 2 min in 1.0 ml of methanol, and subjected to three sonication cycles of 30 min each in an ultrasound bath. The resulting mixture was then filtered using 0.45 μm membrane, and CysA was then quantified by HPLC. It was observed that the sonication step did not affect the stability of CysA.

The concentration of drug in dermal was an index of topical delivery, whereas the concentration in receptor phase was an index of transdermal delivery.

### 2.4. Ultrasound application

Low-frequency ultrasound was applied with a sonicator (Beijing Medical Treatment Instrument Corporation, China) with a transducer. The radiating diameter of transducer was 10 mm. The frequency was set as 20 kHz and a 50% duty cycle (1 s 'on' and 1 s 'off'). The ultrasound transducer was located approximately 0.5 cm from the surface of rat skin. The sonicator was 'turned' before each new experiment according to a procedure specified by the manufacturer to ensure that the signal applied optimally matches the resonance frequency of piezoelectric crystal. During the experiments, the influences of two sonication parameters were studied: intensity during the 'on' period and total exposure time.

### 2.5. Combination effect of ultrasound and chemical enhancers

Menthol (10%, w/w), SLS (0.05%, w/w), Azone (5%, w/w), NMP (5%, w/w) and DMSO (5%, w/w) were used as chemical enhancers. These enhancers were dissolved in ethanol except SLS (in water). The skin was pretreated by placing 500 μl of the pretreatment solution in the donor compartment of the diffusion cell. The enhancer solution was removed after 2 h of incubation, the remaining enhancer on the surface of skin was eliminated. CysA suspension at a concentration of 0.5% was added to the donor compartment, and then the ultrasound was immediately irradiated to the compartment for 30 min. The ultrasound intensity was set at 0.8 W/$cm^2$.

### 2.6. Combination effect of ultrasound and electroporation

Electroporation was performed using an exponential decay pulse generator (Beijing Medical Treatment Instrument Corporation, China). The Ag–AgCl electrodes were used. The anode was positioned in the donor compartment, while the cathode was positioned in the receptor compartment. Electroporation protocol was 1 pulse per 20 s (3 ppm), applied for 20 min. The pulse voltage was 110 V and pulse length was 300 ms. Voltages were expressed as applied values but not transdermal values. After 20 min of electroporation of skin, the low-frequency ultrasound (0.8 W/$cm^2$, 30 min) was then applied.

### 2.7. Combination effect of chemical enhancers and ultrasound and electroporation

After the completion of chemical enhancer pretreatment procedure as described above, CysA solution at a concentration of 0.5% was added to the donor compartment, and then the electroporation (110 V, 300 ms, 3 ppm) was immediately irradiated to the compartment for 20 min. Finally, ultrasound was applied for 30 min. The frequency was set at 20 kHz, and the intensity was 0.8 W/cm$^2$.

### 2.8. Analytical methodology for CysA

CysA was analyzed by reversed phase HPLC using JASCO 1500 series. The column was a Diamond $C_{18}$ column (5 μm, 4.6 mm × 200 mm). The samples were chromatographed using a mobile phase consisting acetonitrile–water–phosphoric acid (750:250:1) at a flow rate of 1.0 ml/min. The detection wavelength was set at 210 nm and oven at 70 °C. The retention time of CysA was 13.5 min. No interference of the other formulation components was observed. All samples filtered through a 0.45 μm pore size membrane filter before injection.

### 2.9. Skin toxicity studies—histopathology of skin

Histological changes in the rat skin were examined after ultrasound. Immediately after pretreatment of ultrasound or combination with ultrasound and Azone, a specimen of the exposed area was taken for histological examination. The adjacent untreated skin area was also assessed as the control. Each specimen was fixed in 10% formaldehyde solution for at least 48 h. The specimen was cut vertically against the skin surface. Each section was dehydrated using ethanol and then embedded in paraffin wax, stained with hematoxylin and eosin. The specimens were evaluated under a light microscopy.

### 2.10. Statistical analysis

All skin permeation experiments were repeated at least four times and data were expressed as the mean value ± S.D. Statistical data were analyzed by one-way analysis of variance (ANOVA). A multiple comparison test was used to compare different formulations, and a $P$-value of 0.05 was considered to be significant.

## 3. Results and discussion

### 3.1. Ultrasound alone

Since we found that 40% ethanol was a potential vehicle for topical delivery of CysA in our previous study (Liu et al., 2006b), 40% ethanol was chosen as the coupling medium.

In the first experiment (see Fig. 1), ultrasound was applied over 30 min using three different intensities (0.4, 0.8 and 1.2 W/cm$^2$). No significant modification in the deposition of CysA into deeper skin was observed when 0.4 W/cm$^2$ intensity as applied. When 0.8 W/cm$^2$ was used, the concentration of the drug in deeper skin was almost seven-fold higher than the control group ($P < 0.05$). However, when the intensity increased to 1.2 W/cm$^2$, the deposition of CysA into deeper skin was slightly reduced compared to the group which the intensity of ultrasound was set at 0.8 W/cm$^2$.

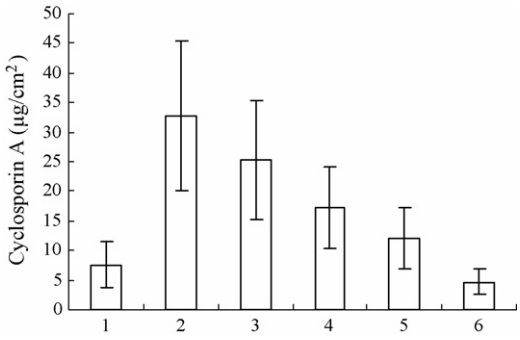


Fig. 1. Amount of Cyclosporin A in rat deeper skin after exposure to ultrasound of various protocols. 1: 0.4 W/cm$^2$, 30 min; 2: 0.8 W/cm$^2$, 30 min; 3: 1.2 W/cm$^2$, 30 min; 4: 0.8 W/cm$^2$, 10 min; 5: 0.8 W/cm$^2$, 60 min; 6: the control. The coupling medium: 40% ethanol. All data represent the means of five experiments ± S.D.

In the second experiment, the dependence of CysA enhancement over the total application time was studied. Fig. 1 also compared the topical delivery of CysA to three different exposure times (10, 30 and 60 min) with all the other ultrasound parameters remaining constant ($I = 0.8$ W/cm$^2$). An intensity of 0.8 W/cm$^2$ was chosen since it allowed the higher permeation of the drug into the deeper skin. The most significant enhancement of CysA permeation was 30 min application time and the application of the highest application time (60 min) increased the amount of CysA retained in deeper skin only 2.57 times than the passive diffusion.

The mechanisms by which ultrasound enhances skin permeability are not yet fully understood. Ultrasound may increase skin permeability for drugs by lowing the barrier properties of the stratum corneum (Machet and Boucaud, 2002; IIana and Joseph, 2004). Cavitation, i.e. the generation and oscillation of gaseous bubbles in medium exposed to ultrasound, has been shown to play a major role in sonophoresis (Tang et al., 2002; Mitragotri and Kost, 2004). Creation of small pores at the skin surface and disorganization of the lipid bilayers within the stratum corneum after ultrasound exposure, have been related to cavitation. Although cavitation is complex phenomenon and depends on various parameters, it can occur more readily with low-frequency ultrasound than with high-frequency ultrasound. Changes in the deposition of CysA into the skin following ultrasound parameters involved in the occurrence of cavitation to explain the skin penetration enhancing effects of sonophoresis.

Intensity is an important parameter for cavitation (Terahara et al., 2002). The relationship between cavitation and intensity is complex. For example, there exists a threshold level of intensity below which cavitation will not occur and it is difficult to obtain significantly more vigorous cavitation by increasing intensity (Tezel et al., 2001). Mitragotri et al. reported that skin conductivity enhancement is proportional to intensity up to 14 W/cm$^2$ (Mitragotri et al., 2000a) (ultrasound frequency: 20 kHz, distance the horn from the skin: 1 cm). However, the linear rela-

Table 1
Effect of various ultrasound protocols on transdermal delivery of Cyclosporin A through rat skin

| | Applied intensity ($W/cm^2$) | Exposure time (min) | Cumulative amount ($\mu g/cm^2 \pm$ S.D.) |
|---|---|---|---|
| 1 | 0.4 | 30 | 1.05 ± 0.32 |
| 2 | 0.8 | 30 | 1.09 ± 0.26 |
| 3 | 1.2 | 30 | 0.93 ± 0.19 |
| 4 | 0.8 | 10 | 0.82 ± 0.11 |
| 5 | 0.8 | 60 | 0.81 ± 0.16 |
| Control | — | — | 0.84 ± 0.28 |

The coupling medium: 40% ethanol. All data represent the means of five experiments ± S.D.

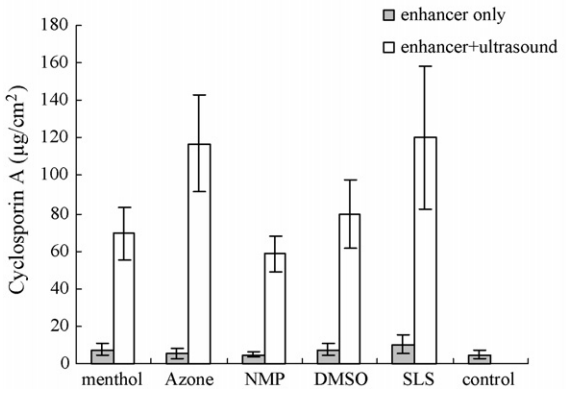


Fig. 2. Amount of Cyclosporin A in rat deeper skin after pretreatment with enhancers alone or in combination with ultrasound (20 kHz, 0.8 $W/cm^2$, 30 min). The coupling medium: 40% ethanol. All data represent the means of six experiments ± S.D.

tionship between skin conductivity enhancement and intensity may break down at higher intensities due to the presence of a cavitation cloud. This cloud is generated near the ultrasound source due to cavitation and reduces amount of energy delivered to the skin. Our results showed that the dependence of the amount of CysA retained in deeper skin enhancement on ultrasound intensity. As the intensity increased, enhancement also increased up to a certain point, then dropped off. The intensity ($I_{max}$) at which enhancement was maximum occurs at about 0.8 $W/cm^2$ for 20 kHz. The increase in enhancement up to $I_{max}$ was attributed to the higher energy levels delivered to the skin and the decrease in the enhancement beyond the $I_{max}$ was due to acoustic decoupling, a process which decreased the intensity 'seen' by the skin due to the presence of cavitation cloud.

In contrast to the results by Boucaud et al. (2002), our results showed that topical CysA transport did not enhance for a given intensity as the total application time increased. The exposure time at which enhancement is maximum occurs at about 30 min. It might be due to the less cavitation by the much more application time. The exactly explanation would be further assessed.

In addition, the amount of CysA in receptor medium did not show any significant increase (see Table 1). The accumulation of CysA into deeper skin by low-frequency ultrasound was much more than the amount of the drug delivered across the skin.

### 3.2. Combined effect of ultrasound and chemical enhancers

Combination of ultrasound and chemical enhancers offers several advantages over the use of ultrasound or chemicals alone, such as its high efficiency and ease of application (Mitragotri, 2000). The amount of CysA retained in rat skin after pretreatment with various enhancers alone and in combination with low-frequency ultrasound was shown in Fig. 2. In absence of ultrasound, the deposition of CysA into deeper skin decreased in the following order 0.05% SLS > 10% menthol > 5%DMSO > 5% Azone > 5% NMP and in presence of ultrasound the deposition of the drug was 0.05% SLS ≈ 5% Azone > 5% DMSO > 10% menthol > 5% NMP. Relative to the passive permeability (measured over 6 h), application of enhancers alone induced up to a two-fold increase in the amount of CysA in deeper skin, whereas application of ultrasound (0.8 $W/cm^2$, 30 min) alone only induced a seven-fold enhancement. However, when combined, the pretreatment with SLS or Azone followed a 30 min application of ultrasound induced about 25-fold increase in the dermal delivery of CysA ($P < 0.01$). Hence, the 25-fold enhancement obtained by a simultaneous application of SLS or Azone and ultrasound was due to their synergistic effect. Mitragotri et al. performed an evaluation of the synergistic effect of low-frequency ultrasound with SLS (Mitragotri et al., 2000b). They suggested that three possible mechanisms played a role in this synergistic effect: SLS enhanced ultrasound-induced cavitation; ultrasound derived more SLS into the skin; ultrasound may enhance the dispersion of SLS in the SC. The latter two mechanisms were found to be dominant. Johnson et al. suggested that ultrasound might 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 favourable molecular arrangement which would remain stable even after ultrasound was turned off (Johnson et al., 1996). Recently, Lavon et al. demonstrated that simultaneous application of ultrasound and SLS led to modification of the pH profile of the SC (Lavon et al., 2005). This pH modification within the SCs microenvironment, could affect both the structure of the lipid layers and the activity of SLS as a chemical enhancer due to its improved lipophilic solubility. The altered pH profile that resulted in improved SLS lipophilic solubility, together with improved SLS penetration and dispersion, can explain the synergistic enhancing effect of ultrasound and SLS on transdermal transport. In this study, ultrasound may be attributed to better distribution of enhancers already delivered into the skin during the initial 2 h soaking. This may allow a larger fraction of lipids to contact the enhancers, thereby increasing the magnitude of disrupted lipids.

Since there is no reported value for therapeutically required concentration of CysA to treatment of autoimmune diseases, we can only make a speculation. Topical delivery (in vitro) of a 0.4% CysA in a lipid mixture containing ethanol in human skin was reported to have resulted in 4.079 $\mu g/cm^2$ in the SC and 0.042 $\mu g/cm^2$ in the deeper skin after 6 h non-occlusive application (Verma and Fahr, 2004). In comparison to that we obtained a significant increased value in the deeper skin combined with the use of ultrasound and skin pre-treatment with Azone or SLS ($P < 0.01$). The accumulation of CysA in mice deeper skin from

Table 2
The amount of Cyclosporin A in receptor medium after pre-treatment with various enhancers alone and in combination with ultrasound (20 kHz, 0.8 W/cm$^2$, 30 min)

| | Cumulative amount of Cyclosporin A in receptor medium (μg/cm$^2$ ± S.D.) | | | | |
|---|---|---|---|---|---|
| | Menthol | Azone | NMP | DMSO | SLS |
| Enhancer | 1.63 ± 0.54 | 1.36 ± 0.41 | 1.25 ± 0.20 | 1.21 ± 0.34 | 1.19 ± 0.19 |
| Enhancer + ultrasound | 1.60 ± 0.39 | 2.37 ± 0.38 | 1.15 ± 0.33 | 1.05 ± 0.17 | 3.14 ± 0.44 |

The coupling medium: 40% ethanol. All data represent the means of six experiments ± S.D.

40% ethanol vehicle was about 30 μg/cm$^2$ by electroporation in vitro (Wang et al., 1998), which was much lower than the result we obtained ($P < 0.05$). Lopes et al. suggested that using monoolein as a penetration enhancer 4% CysA in propylene glycol formulations could promote the topical delivery up to about 140 μg/cm$^2$ in the SC and 30 μg/cm$^2$ in the deeper skin in vitro using porcine ear skin at 12 h-post application (Lopes et al., 2005). Concerning the dose of the drug used by Lopes et al. was much higher than that of we used, combination of ultrasound and chemical enhancers may be an appropriate vehicle for the topical delivery of CysA. Our approach can be effective for topical treatment of inflammatory skin diseases like psoriasis, atopic dermatitis, and some hair follicle disorders.

Table 2 compared the amount of CysA in receptor medium with enhancer pretreatment alone and in combination with low-frequency ultrasound. After skin pretreatment with enhancers, the transdermal delivery of CysA was slightly promoted. When combined with Azone or SLS and ultrasound, the cumulative amount of CysA in receptor medium was 2.82 and 3.74 times than the passive diffusion, respectively. Nevertheless, the accumulation of CysA through skin by combination with enhancer and ultrasound was much less than the amount of drug delivered into the deeper skin. These results suggested that the combination strategy increase the topical delivery of the drug, but had no obvious effect on the transdermal delivery.

### 3.3. Combination effect of ultrasound and electroporation and enhancer

Transdermal electroporation is the application of short (1 s), high voltage (50–500 V) pulses to the skin to cause disorganization the stratum corneum lipid structure and enhance drug delivery. Electroporation enhances transdermal transport through enhanced diffusion (via skin poration), electrophoresis, and electroosmosis (Dimitrov and Sowers, 1990; Klenchin et al., 1991; Prausnitz et al., 1995; Edwards et al., 1995). Kost et al. investigated the synergistic effect of therapeutic ultrasound and electroporation on transdermal transport of two molecules, calcein and sulforhodamine (Kost et al., 1996). Fig. 3 showed the influence of combination of enhancer and ultrasound and electroporation on the amount of CysA retained in the skin. The result demonstrated that ultrasound combined with electroporation was not effective in enhancing the topical delivery of CysA. When applied simultaneous with ultrasound and Azone and electroporation, the amount of CysA retained in the skin was higher than that of ultrasound alone but also lower than that of combination with ultrasound and Azone. Fig. 3 also showed the effect

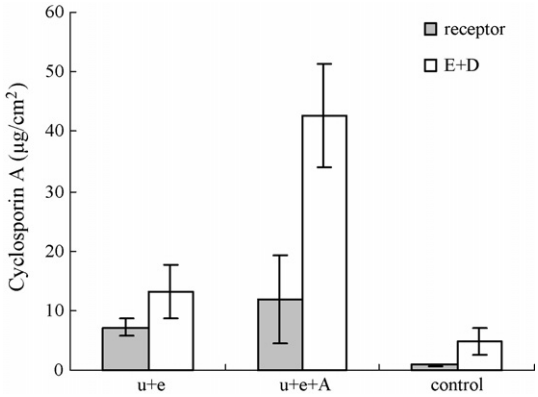


Fig. 3. Effect of combination with chemical/physical enhancers and ultrasound (20 kHz, 0.8 W/cm$^2$, 30 min) on topical (E + D, epidermal + dermal) and transdermal (receptor) delivery of Cyclosporin A. u: Low-frequency ultrasound; e: electroporation; A: Azone. The coupling medium: 40% ethanol. All data represent the means of four experiments ± S.D.

of combination of ultrasound (20 kHz, 0.8 W/cm$^2$, 1 s on and 1 s off) and electroporation (110 V, 300 ms, 3 ppm) and Azone pretreatment on the transdermal transport of CysA. Application of ultrasound or electroporation alone did not markedly enhance transdermal delivery of CysA for 6 h (1 and 2.5 μg/cm$^2$, respectively), while a simultaneous application of ultrasound and electroporation enhanced transdermal CysA transport to 7 μg/cm$^2$ for 6 h. Trimodality treatment comprising of pretreatment with Azone + ultrasound in combination followed by electroporation enhanced transdermal CysA transport to 12 μg/cm$^2$. CysA is a neutral, lipophilic undecapeptide and is not known to exist as a zwitterion. Thus, the enhanced transdermal delivery of CysA may predominantly attribute to the creation and/or the enlargement of aqueous pathways during electroporation. The synergistic interaction of ultrasound and electroporation might be caused by ultrasound partially disorganizing stratum corneum lipids, thereby making them more susceptible to electroporation. The enhanced transdermal delivery of CysA might initiate side effect.

### 3.4. Histopathology of skin

At the end of the study, skin biopsies were examined microscopically and histological scores (quantifying the damage caused the application) were calculated. Histological assessment allows one to quantify the damage caused to the epidermis and SC, such as swelling of the individual cells (intracellular oedema). The result (Fig. 4) did not reveal any lesions after application of low-frequency ultrasound or combination with

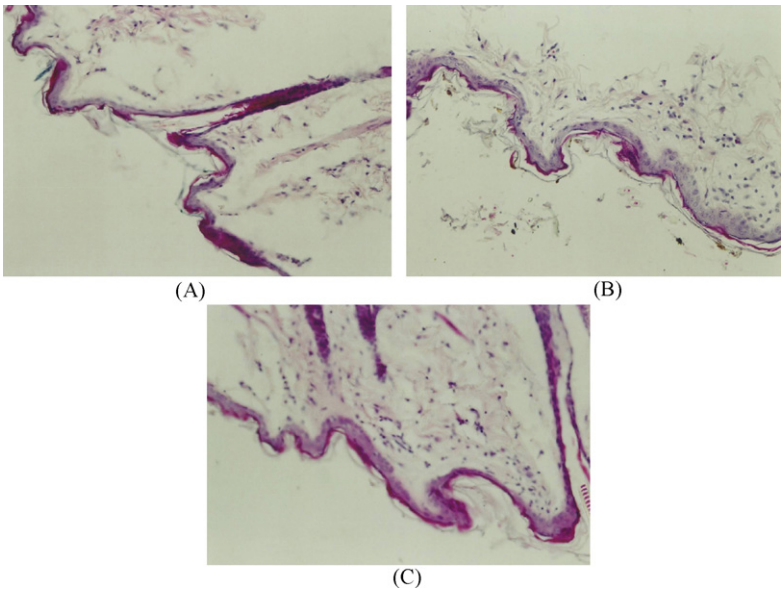


Fig. 4. Histological photomicrographs of skin biopsies taken from rat treated with various applications. H&E stain. (A) Untreated rat skin. (B) Rat skin treated with ultrasound (20 kHz, 0.8 W/cm$^2$, 30 min). (C) Rat skin treated combination with ultrasound and Azone.

ultrasound and enhancers. The stratum corneum and viable epidermis were of normal appearance.

## 4. Conclusion

From the results, it can be concluded that the topical delivery of Cyclosporin A can be enhanced by low-frequency ultrasound. The enhancement was dependent on ultrasound intensity, exposure time and pretreatment with chemical or physical enhancers. Combination ultrasound and SLS or Azone lead to maximal enhancement in topical delivery of the drug. Trimodality treatment comprising of pretreatment with Azone + ultrasound in combination followed by electroporation was not effective in enhancing the topical delivery of Cyclosporin A, but could increase the penetration of Cyclosporin A through rat skin by order of 15. The synergistic effect of ultrasound and combination of chemical enhancer may permit the use of lower quantity of chemical enhancer and intensity within the delivery system for achieving therapeutically effective topical drug concentration.

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