Phonophoretic Transdermal Drug Delivery of Triamcinolone acetonide gel

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트리암시놀론 겔의 음파영동 경피약물흡수

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국문초록

스테로이드성 소염진통제인 트리암시놀론 겔의 피부투과도를 향상시키기 위하여 초음파를 조사하여 약물의 투과도에 미치는 영향을 비교하였다.

트리암시놀론을 함유한 수용성 겔을 제조하여 물리화학적 시험을 실시하였으며 carbopol을 기제로한 겔이 우수한 제제학적 특성을 보였다.

초음파 조사가 약물의 투과도에 미치는 영향을 알아보기 위하여 hairless mouse의 적출 피부에 대한 *in vitro* 흡수 실험을 실시하였다. 트리암시놀론 겔 음파영동군이 트리암시놀론 겔 단독 처치군에 비하여 투과도가 유의적으로 향상되었다. 특히 주파수가 1㎒인 지속초음파를 고 강도로 적용시 피부투과도의 향상이 더욱 두드러졌다.

따라서 트리암시놀론 겔 도포 후 초음파를 이용한 음파영동 경피흡수가 단독의 겔 처치보다 피부투과에 유용할 것으로 사료된다.

Ⅰ. Introduction

The skin application of drugs has a lot of advantages, but there is a restriction to generalize it in that it has no certain solution to the low skin permeability of drug. In case of skin application of drug it is important to improve chemical permeability and skin permeability by reducing the function of intestinal walls of horny layers. Skin permeation enhancer has been used to increase skin absorption. As a skin absorption method by physical agents, iontophoresis making use of direct current has been widely used (Riviere and Heit, 1997). The important problems with iontophoresis are that it has a risk to burn skins because of the change of pH and the increase of current density in applying it and that it can be applied only in the form of water solution (Ahn, 1991). As another method, phonophoresis by ultrasound has several advantages that it has a low risk to burn skins, and it, unlike iontophoresis, is not necessary to ionize drugs, and its permeability is about 5 ㎝ deep and its treatment time is short (Tyle and Agrawala, 1989; Mitragotri *et al.*, 1996, Yong *et al.*, 2000).

Transdermal drug delivery has attracted considerable attention in recent years and

the potential advantages of this mode of administration have been well documented (Guy and Hadgraft, 1988). Transdermal delivery has attractive advantages such as avoidance gastrointestinal degradation and hepatic first-pass effect, a controlled sustained delivery system and augmentation of patient compliance, since a transdermal formulation would be easy to apply and remove (Cullander and Guy, 1991).

Triamcinolone acetonide is a synthetic fluorinated corticosteroid. The drug occurs as white to cream-colored, crystalline powder having not more than slight odor and practically insoluble in water and very soluble in alcohol. *Triamcinolone acetonide* shares the actions of the other topical corticosteroids and is used for the relief of the inflammatory manifestation of corticosteroid-responsive dermatitis. The drug is also used as a paste for adjunctive treatment to provide temporary relief of symptoms associated with oral inflammatory or ulcerative lesions resulting from trauma.

Dermatological preparations of triamcinolone acetonide are applied. Sparingly in very thin films are rubbed gently into the affected area 2-4tines daily. The 0.5% cream and 0.5% ointment should be used only in the treatment of dermatitis which are refractory to treatment with % were concentrations.

This study is to enhance drug penetration via skin following adoption of ultrasound. For this goal, in gel containing triamcinolone acetonide, the degree of skin penetration in vitro.

II. Materials and Methods

1. Materials and instruments

Triamcinolone acetonide was purchased from Sigma Chemical Co., (St. Louis. MO, USA). All other chemicals were of reagent grade and used without further purification.

Male hairless mice (25 ± 2.5 g) were purchased by Dae-Han experimental animal center (Daejeon, Korea).

Ultrasound was applied at frequencies of 1 or 3㎒, and intensities of up to 2 W/㎠ using an ultrasound generator(model Sonopulse 590, Enraf-Nonius, Netherlands).

The amount of Triamcinolone acetonide were quantified using a HPLC system (LC-10AT, Shimadzu, Japan). Skin permeability of triamcinolone acetonide was measured by the skin permeation tester (DST 600A, Fine Scientific Co., Seoul, Korea).

2. Solubility of Triamcinolone acetonide

The solubility of triamcinolone acetonide was determined in various phase.

An excess amount of triamcinolone acetonide was added into 2.0 mL of each dissolution medium and then the mixture was stirred for 72 hours at room temperature. Triplicate samples were centrifuged at 3,000 rpm for 10 min to remove

the excess amount of drug undissolved. Then, aliquot of supernatant was taken and the content of drug was assayed by HPLC system following dilution with methanol.

3. Determination of triamcinolone acetonide by HPLC system

The amount of Triamcinolone acetonide were quantified using a HPLC system consisting of solvent delivery system, C_{18} reverse column (WAT 027324, Waters Co., Milford, Massachusetts, USA), multi-wavelength detector and integrator

The detailed conditions of HPLC system were following;

Column : μ-Bondapak C_{18} (3.9×300mm)

Mobile phase : the mixture of acetonitrile : water: formic acid (40.0 : 59.9 : 0.1)

Flow rate : 1.2 mL/min

Injection volume : 10 ㎕

Detector : UV detector (λ_{max} 238 ㎚)

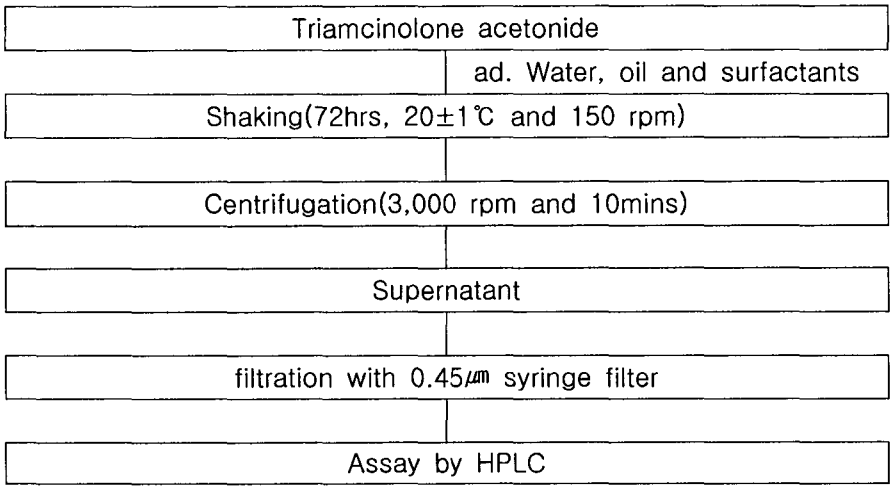

Fig. 1. Procedure of determine triamcinolone acetonide solubility

4. Skin permeability of Gel

When applying triamcinolone acetonide from gel 1 mL to the skin, Franz diffusion cell was used it was applied the material made above in donor phase, while in receptor phase and filled with each 12.0 mL of pH 7.4 phosphate buffer : 20% Transcutol (1:1) solution, fixed it with clamp after setting the skin of hairless mouse, and maintained the temperature of 35。C. At this time, the effective expansion area contacting with receptor phase was 1.77 ㎠.

Every designated time, after gathering 0.1 mL of aqueous solution correctly, and diluted with the same amount of methanol, and tested after filtering with 0.45 ㎛ membrane filter, supplemented the same amount of fresh saline in aqueous solution.

The accumulated amount of triamcinolone acetonide administered per unit area of the skin was represented with function corresponding time. Lag-time method was used to investigate dynamic state of drugs through the skin. Using the under formula, we calculated diffusion integer, that is, distribution coefficient between skin and base, which equals transmission speed at the equilibrium state.

$$Js = \frac{1}{A}\left(\frac{dQ}{dt}\right)ss = \frac{DKC}{h}$$

$$D = \frac{h^2}{6T_L}$$

Only,

Js = transmission speed at the equilibrium state,

A = transmission membrane area,

(dQ/dt)ss = the amount of drugs passing the membrane per unit time at the equilibrium state,

C = concentration of drugs in donor compartment,

K = distribution coefficient of drugs,

h = thickness of membrane,

D = diffusion integer of drugs through skin,

T_L = lag-time

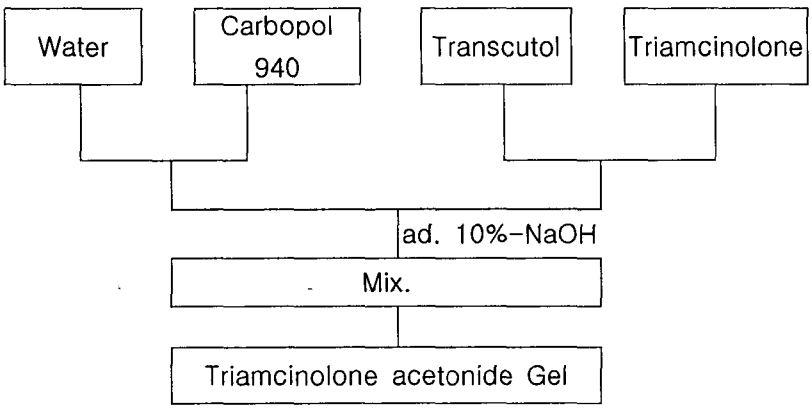

Fig. 2. Preparation of triamcinolone acetonide gel

Table 1. Preparations of gel containing triamcinolone acetonide with different content of drug(g).

Preparation	Gel A	Gel B
Triamcinolone	0.10	0.01
Carbopol 940	0.08	0.08
10%-NaOH	0.20	0.20
Transcutol	3.00	3.00
Water	6.62	6.71
Total	10.00	10.00

III. Results

1. Determination of triamcinolone acetonide

High performance liquid chromatography method was developed for the determination of triamcinolone acetonide, and typical chromatograms are showed (Figure 3). The detection of limits for triamcinolone acetonide was 0.1㎍/mL. A good correlation between the response and concentration could be obtained without using internal standard. Figure 4 shows standard calibration curve of triamcinolone acetonide.

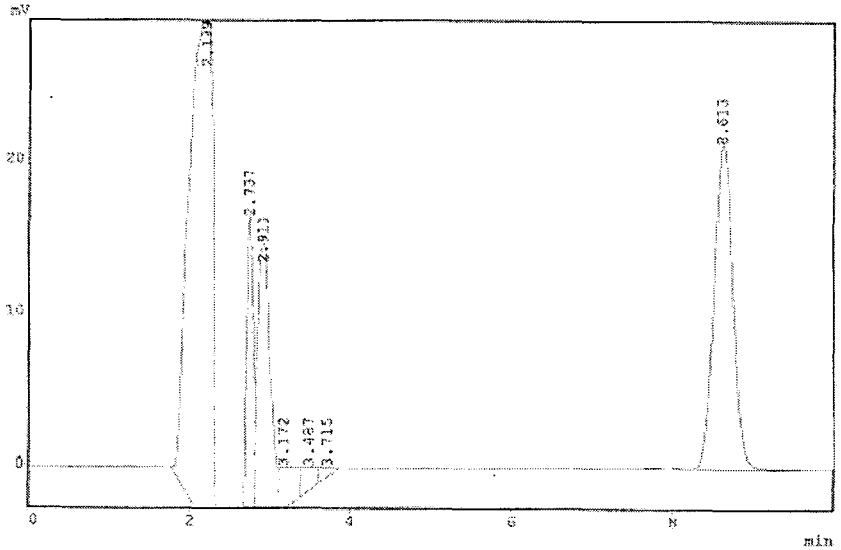

Fig. 3. Chromatogram of triamcinolone acetonide

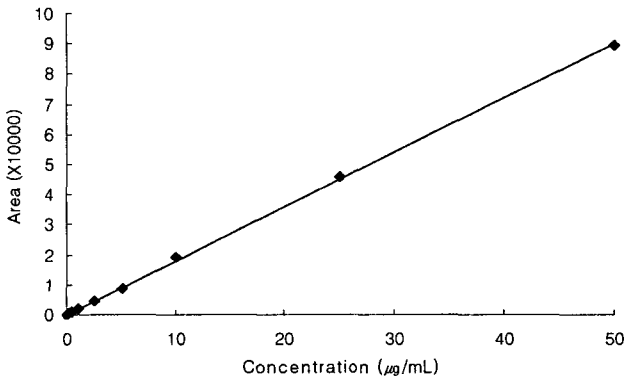

Fig. 4. Calibration curve of triamcinolone acetonide by HPLC
y= 27888.0646 + 19253.4023 x r^2= 0.9998

2. Solubility of triamcinolone acetonide

The solubility of triamcinolone acetonide in each component was 67.97 ±0.51 mg/mL in Transcutol, 24.70 ± 1.15 mg / mL in Labrasol and 62.21 ± 4.31 mg/mL in dimethylisosorbid, respectively. To determinate the penetration rate through the skin, 10% of Transcutol was added to the phosphate buffer as receptor phase (Table 2).

Table 2. Solubilities of triamcinolone acetonide in water, oils, and surfactants

Solvents	Solubility (mg/mL)
Water	0.13 ± 0.02
Triacetin	8.21 ± 2.37
Labrasol	24.70 ± 1.15
Dimethylisosorbid (DMI)	62.21 ± 4.31
Transcutol	67.97 ± 0.51

An excess amount of triamcinolone acetonide was added into 3 mL of each dissoultion medium and stirred for 48 hrs at room temperature. The content of drug was assayed by HPLC
Each data represents the mean ± SE from 5 experiments

3. Stability of gel

According to the variation of triamcinolone acetonide concentration of produced

gel, for 6 months at 4℃, 37℃ and 50℃. It could be secured stability because of no change significant of the triamcinolone acetonide concentration in all prescriptions for six months.

4. Skin permeation of triamcinolone acetonide

Figure 5 illustrates the effect of triamcinolone acetonide concentration affecting drug administration through extracted hairless mouse skin. Following adoption of ultrasound both 1㎒ and 3㎒, it showed relatively high permeation rate where it was compared with non treated by ultrasound. The influence of frequency having an effect on skin permeation rate was the highest in the case of using 1㎒ (Figure 6) and continuous mode treatment (Figure 7).

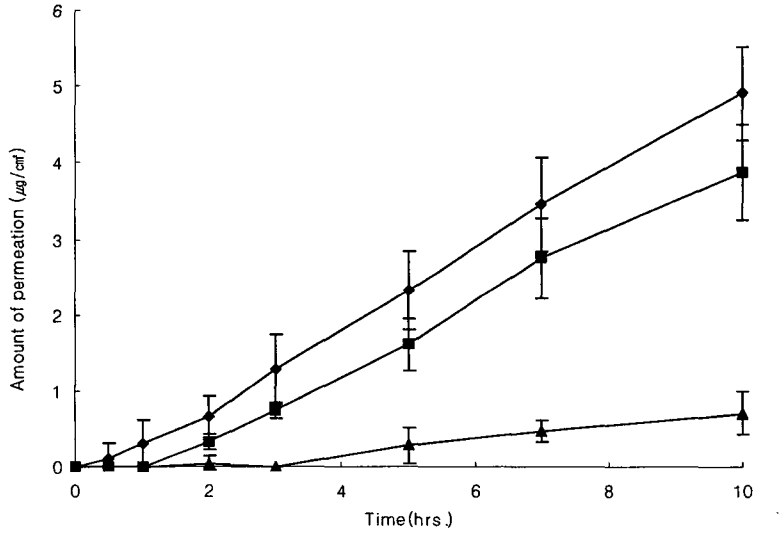

Fig. 5. Effects of triamcinolone acetonide contents in cumulative skin permeation through excised hairless mouse skin
◆ : Gel A, ■ : Gel B, ▲ : ointment (commercial)
Each bar represents the mean ± SE from 5 experiments

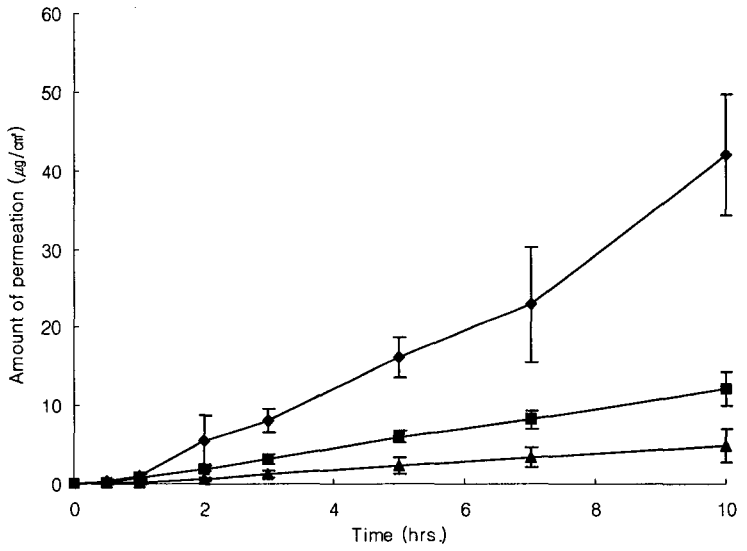

Fig. 6. Effects of various frequency in cumulative skin permeation through excised hairless mouse skin

◆ : 1 MHz, ■ : 3 MHz, ▲ : none ultrasound

Each bar represents the mean ± SE from 5 experiments

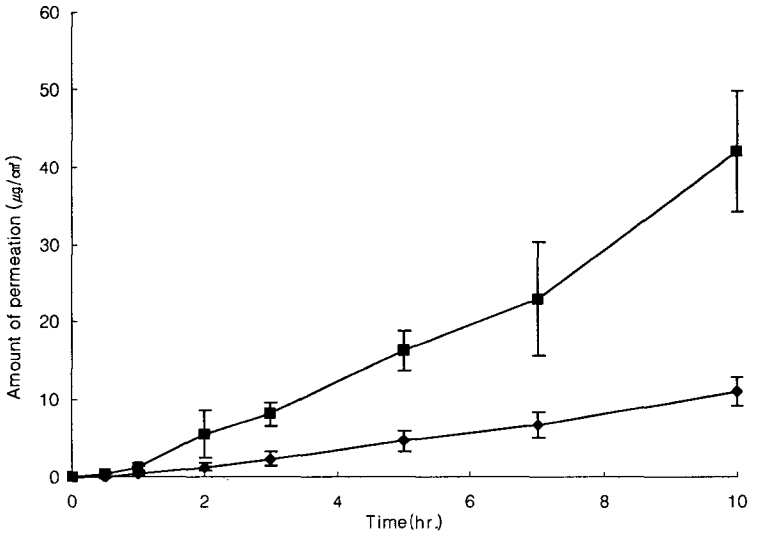

Fig. 7. Effects of continuous and pulse treatment in cumulative skin permeation through excised hairless mouse skin

◆ : pulse, ■ : continuous

Each bar represents the mean ± SE from 5 experiments

Figure 8 illuminate the effects of temperature experiment result of skin permeation in gel containing triamcinolone acetonide. Drug skin permeation was increased constantly to hours. The influence of various medium temperature affecting skin permeation was the highest at 40℃, but it was no significant difference. Skin permeation increase attended by intensity of ultrasound, the permeation of trice was accelerated at 2.5 w/㎠ than 1.0 w/㎠ (Figure 9), (Table 3).

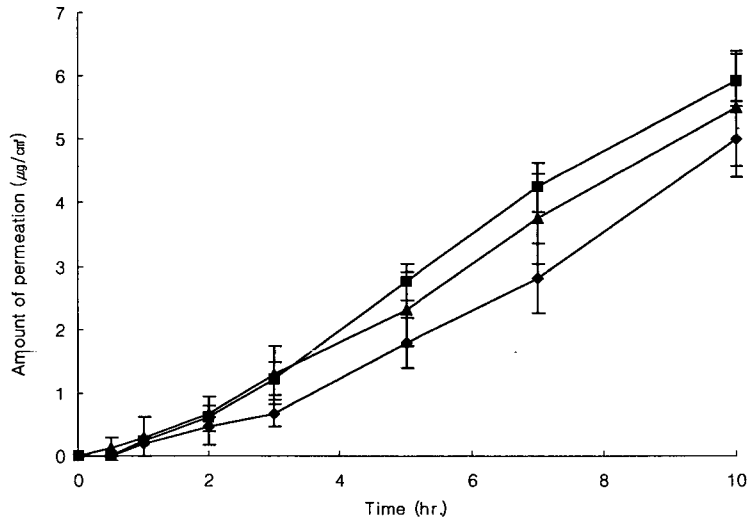

Fig. 8. Effects of temperature in cumulative skin permeation through excised hairless mouse skin

◆ : 30 ℃, ▲ : 35 ℃, ■ : 40 ℃

Each bar represents the mean ± SE from 5 experiments

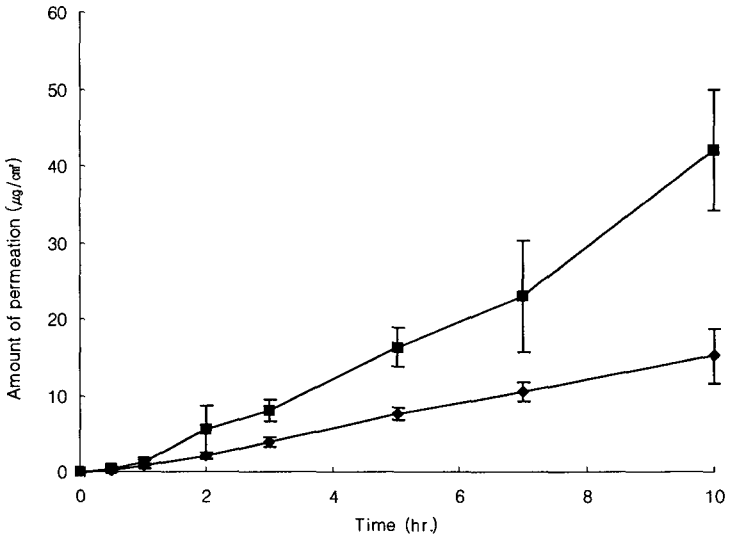

Fig. 9. Effects of intensity as water phase in cumulative skin permeation containing triamcinolone acetonide through excised hairless mouse skin
◆ : 1.0 w/㎠, ■ : 2.5 w/㎠
Each bar represents the mean ± SE from 5 experiments

Table. 3. Permeation parameters of Triamcinolone acetonide gel through excised hairless mouse skins

Application	Parameters		
	Js	T_L	AUC
A	0.4726±0.2013	0.416±0.275	23.361±9.727
B	1.1749±0.1958	0.386±0.142	57.589±9.075
C	0.9812±0.2168	1.315±0.643	47.392±11.281
D	1.4574±0.2676	0.518±0.329	72.198±11.817
E	3.5965±0.8056	1.295±0.483	171.377±40.495

Application A : Non-Ultrasound
Application B : 3 MHz, continuous
Application C : 1 MHz, pulse, 2.5w/㎠
Application D : 1 MHz, continuous, 1.0w/㎠
Application E : 1 MHz, continuous, 2.5w/㎠
Js : Permeation rate (㎍/㎠/hr)
T_L : Lag-time (hr)
AUC : Area under the concentration (㎍/㎠)

IV. Discussion

When sound waves are divided by frequency ranges, ultrasound is a sound wave beyond the audible sound waves between 20 - 20.000 Hz. Pierre Curie found out that polycrystalline material like crystal generate electric charge outside depending on the change of pressure, and he called it a piezo-electric effect. When this phenomenon happens in bone tissues, collagen fibers, and body protein, ultrasound is a cause that makes the biological effects happen (Hoogland, 1991). A reverse piezo-electric effect is a principle by which ultrasound is generated by passing polycrystalline materials through high frequency alternating current and causing the vibration of contraction and extension in high frequency. The physical characteristics of ultrasounds are reflection, refraction, scattering, absorption and penetration against medium.

Studies on ultrasound and a living body were substantially started in the 1920s when it was found out that high pressure waves generated by ultrasound in the water injured tissues of a living body. It was in the 1930s that ultrasound started to be used to cure diseases. The biophysical effects of ultrasound are generated by the mechanical effects resulting from microvibration or micromassage in the process that ultrasound passes through a living body. One of the mechanical effects of ultrasound is the cavitation phenomenon that is formed in the process that small air bubbles are constricted and expanded in tissue fluids and its blood by the vibration of ultrasound. While stable cavitation increases the membrane permeability of cells and its activity, unstable cavitation damages tissues. Also the eddy currents produced by cavitation develop the tissue fluids activity by making rotational forces and stresses given to intracellular organelles existing near vibrating gas bubbles (Nyborg, 1982). Consequently, it causes acoustic streaming effect that increases membrane permeability, ion flexes, and cells' activity by flow the tissue fluids into one direction under the influence of ultrasound beams. These mechanical effects become the greatest in high intensity, low frequency and continuous mode (Nussbaum, 1996). These mechanical effects make a change of about 0.02% in the volume of cells, change membrane permeability of cells and tissues, and increase the exchange of metabolic products. If the tissue's weak microvibration by ultrasounds increase, it can produce a thermal effect by inducing the generation of friction heat to increase tissue's thermometer. The biological changes made by the mechanical and thermal effects of ultrasound includes promotion of blood circulation, increase of tissues' regenerative power, increase of membrane permeability, improvement of tissue circulation, effects on peripheral nerves, and muscle relaxation, reduction of pain (Dinno *et al.*, 1989).

Phonophoresis as drug delivery systems to attempt a design of new dosage forms has been investigated with gel containing triamcinolone acetonide. Gel formulation was successfully manufactured and which was stable enough during the experiment.

Cabopol was usually used for hydrophilic gel preparations. Cause low solubility of triamcinolone acetonide, Transcutol was used as surfactant but it's concentration was too high. It could be consist that we tried to evaluate the effects of ultrasound.

The transdermal permeation enhancing effects of ultrasound was evidence as results of *in vitro* studies. The temperature of receptor phase was not influenced in skin permeation by phonophoresis. It is shown that temperature and stirring can cause flux enhancements, and orders of magnitude of the corresponding flux increases are consistent with the values observed experimentally. But these two factors are not sufficient to explain the phenomenon. A satisfying microscopic physical interpretation of the supposed decrease in the donor solution-membrane interfacial potential energy barrier, caused by ultrasound. But influence of temperature was not different significant enter 30, 35 and 40 ℃. In several references, the medium temperature was set to 32 ℃ and we also same temperature during the experiments.

Taking advantage of the reproducibility and of the well characterized permeability characteristics of the membranes, various hypotheses have been tested in experiments as an attempt to explain the mechanism of phonophoresis. Temperature increase caused by heat liberation, which would increase the permeability of the drug inside the membrane. Reduction of the boundary layer thickness (close to the membrane), which creates an additional resistance to drug transport, by mixing of the solutions. Radiation pressure, in which the sound wave would exert a pressure on the drug molecules, or on the skin. Decrease of the donor solution-membrane interfacial potential energy barrier. Cavitation is thought to create bubbles that collapse, thus generating shock waves.

The influence of frequency having an effect on skin permeation rate was higher in the case of using 1㎒ and continuous treatment. Continuous phase could be increased the temperature of skin. Hence frequency was also to be connected with the depth of vibration, our results was logic. Skin permeation increase attended by intensity of ultrasound, the permeation of triamcinolone acetonide was accelerated at 2.5 w/㎠ than 1.0 w/㎠.

For a long time, in the clinical iontophoresis and phonophoresis have been widely used as methods to improve the skin absorption for hydrocortisone and dexamethasone and medicines belonging to glucocorticoids, which are anti-inflammatory agents to treat the inflammation of skin and subcutaneous tissues and musculoskeletal inflammation. Especially, the use of phonophoresis for glucocorticoids continues to be growing steadily in the clinic in that it minimizes the tissue injury, applies in the type of cream and gel, and give more comfortable than iontophoresis (Saal, 1993). Indication (Diseases for which a drugs is efficacious), as different neuromuscluoskeletal inflammatory conditions, include muscle contusion, sprain and strain, epicondylitis, tendinitis, bursitis, capsultis, arthritis, and neuritis.

One of the most important effects of glucocorticoids for neuromuscluoskeletal

inflammatory conditions is a strong anti-inflammatory power. These drugs control composition of pro-inflammatory substances such as prostaglandins (PGs) and leukotrienes (Lewis *et al.*, 1986). They prevent the migration of scavenger white blood cells to inflammatory parts, have an ability to stabilize lysosomal membrane inside the injured cells, and attenuate autologous cell destruction by preventing secretion and rupture.

The effect of glucocorticoids is excellent to reduce inflammatory symptom, but it has many side effects. A major effect of them is breakdown by catabolism for collagenous tissues (Oikarinen *et al.*, 1988), and in the case that the medicine is given for a long time, endogenous production is controlled by adrenocortical suppression (Zora *et al.*, 1986). This side effect appears if it is systemically given in high dosage over a long time. The advantage of the absorption method by phonophoresis is that this danger decreases because it is applied to specific local tissues in relatively low dosage.

V. Conclusion

To investigate transdermal delivery system for triamcinolone acetonide following adoption of phonophoresis, hydrophilic gel containing triamcinolone acetonide was prepared with Carbopol®. First, Skin permeation of triamcinolone acetonide was evaluated under various factor, temperature, frequency, intensity etc. Finally the variation of plasma ingredients in rat were also compared with cream preparation.

We can conclude that;

1. The solubility of triamcinolone acetonide was higher in Transcutol® as 67.97± 0.51 mg/mL.
2. The influence of frequency having an effect on skin permeation rate was higher in the case of using 1㎒ and continuous mode treatment.
3. The temperature of receptor phase was not influenced in skin permeation by phonophoresis.
4. Skin permeation increase attended by intensity of ultrasound, the permeation of triamcinolone acetonide was accelerated at 2.5w/㎠ than 1.0w/㎠.

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