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Anti-inflammatory effects by transdermal application of triamcinolone acetonide gel using phonophoresis in rats

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Abstract

The present study was carried out to determine the feasibility of using gel formulations for the transdermal delivery of triamcinolone acetonide (TA), which is one of the synthetic glucocorticoids, in conjunction with phonophoresis, and to develop the carbopol gels of TA. For this purpose, the anti-inflammatory effects of the gel containing TA after the adoption of ultrasound were evaluated by investigating the in vivo change in the serum creatine phosphokinase (CPK) and histological findings. Following a muscle injury, the serum CPK activity decreased significantly in the TA gel group with phonophoresis, comparing with that in the control group and the commercial gel group given ultrasound.

In the gross finding, after a muscle injury, the TA gel group with phonophoresis showed rapid moderation of the injury compared with the three other groups. The histological findings showed that the inflammation was relieved within 72 h after the injury from the TA gel group with phonophoresis. These effects were considerably higher in the phonophoresis group than in the other three groups. Overall, a TA gel using phonophoresis might be used as a new transdermal delivery technique providing enhanced anti-inflammatory effects.

Keywords: Triamcinolone acetonide; Phonophoresis; Transdermal application; Ultrasound treatment; Anti-inflammatory effects

1. Introduction

Triamcinolone acetonide (TA) is one of the long acting synthetic glucocorticoids and has been widely used to treat inflammatory disease (Fallers and Petraco, 1982; Oliver, 1982). It is absorbed rapidly from the gastrointestinal tracts achieving the maximum triamcinolone acetonide levels after 1 h, and oral bioavailability averaged approximately 23% (Derendorf et al., 1995). However, TA is destroyed rapidly as it passes through the liver, which makes it relatively ineffective when administered orally. Therefore, in order to reduce

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the adverse reactions such as gastric disturbances as well as the initial hepatic effects that might occur when orally administered, it is desirable to administer the drug via non-oral routes such as topical, buccal and inhalation.

Of the many drug delivery systems, there are many reports (Reinhold and Haus, 1989; Garren et al., 1989; Harris and Robinson, 1992; Vyas and Jain, 1992; Shin et al., 2000) on transdermal drug delivery. Over the last few decades, transdermal dosage forms have been introduced to provide enhanced delivery via the skin into the systemic circulation using penetration enhancers (Barry, 1983; Shin and Kim, 2000), prodrugs (Waranis and Sloan, 1987) and superfluous vehicles (Kondo and Sugimoto, 1987). Because NSAIDs are administered for an extended period, the current resurgence of interest in the transdermal administration as a route for systemic drug delivery using phonophoresis is a logical approach for increasing the rate of drug permeation across the epithelium.

Phonophoresis by ultrasound has several advantages. It has a low risk of burning the skin, is not necessary to ionize the drugs, and its permeability is approximately 5 cm and its treatment time is short (Tyle and Agrawala, 1989; Mitragotri et al., 1996; Yong et al., 2000). The biological changes made by the mechanical and thermal effects of ultrasound includes the promotion of blood circulation, an increase in the tissues' regenerative power, an increase in the membrane permeability, an improvement in tissue circulation, change of peripheral nerve conduction velocity, and muscle relaxation, reduction of pain (Dinno et al., 1989).

The morphological and functional characteristics of the skeletal muscle are altered by the physiological processes, such as external elements including exercise, as well as nerve, tendon, and muscle injuries (Oh, 1999). Muscle injury is caused by various types of mechanical trauma, such as crush injury (Jarvinen and Sorvari, 1975), accidental massive injury, and long-term violent exercise, and pan-necrosis as a result of the ischemic muscle injury (Hanzlikova and Gutmann, 1979; Cheun, 1995). Phagocytic cells appear within a few hours after a muscle injury, and lymphocytes moves around the injured blood vessel and the phagocytic activity increases. The injury by these microtraumas has a large influence on creatine phosphokinase (CPK) and WBC, neutrophil, myoglobin, and malon dialdehyde levels (Zhang et al., 1998). TA shares the actions of the other topical corticosteroids and has been used to relieve the inflammatory manifestation of corticosteroid-responsive dermatitis. The drug is also used as a paste in an adjunctive treatment to provide temporary relief of symptoms associated with oral inflammatory or ulcerative lesions resulting from trauma.

In a previous paper (Yang et al., 2005), the ultrasound application conditions such as frequency (1.0, 3.0 MHz), intensity (1.0, 2.5 W/cm^2), and the treatment method (continuous, pulse) were determined with 0.5% TA gel. The ultrasound application condition showing the highest permeation was also established using an ultrasound frequency of 1.0 MHz, an intensity of 2.5 W/cm^2, and a continuous output (Yang et al., 2005). Phonophoresis was a new transdermal delivery system for TA with enhanced skin permeability.

This study examined the feasibility of the transdermal delivery of TA from a gel formulation using phonophoresis, and to develop the carbopol gels of TA. For this purpose, the anti-inflammatory effects of the gel containing the TA following the adoption of ultrasound were evaluated by investigating the in vivo change in the serum creatine phosphokinase and histological findings.

2. Materials and methods

2.1. Materials and instruments

Triamcinolone acetonide was purchased from the Sigma Chemical Co. (St. Louis, MO, USA). The commercial gel for ultrasound used in the group II was Progel-2 (Dayo medical Co., Korea). The Harri's hematoxylin solution (Yeongdong Pharm. Co., Seoul, Korea), eosine Y solution (Sigma Diagnostics Accustain, USA), gentian violet solution (1%), and Transcutol (Junsei, Japan) of reagent grade were also used. All other chemicals were of reagent grade and were used without further purification.

HPLC system (LC-10AT, Shimadzu, Japan), ultrasound unit (Sonopulse 590, Enraf-Nonius, The Netherlands), light microscopy (Olympus BX50, Olympus Optical Co. Japan), rotary tissue processor and the tissue embedding console system (Tissue-Tek, Sakura, Japan), UV-spectrophotometer (Hitachi 7060 Auto analyzer, Japan) were used.

2.2. Preparation of triamcinolone acetonide gel

The carbopol hydrogel containing 0.8 (v/v)% TA was prepared using the method reported previously (Yang et al., 2005). Briefly, 0.8 g of carbopol 940 was dissolved in 50 ml of water and the pH of the gel was adjusted using 2 ml of a 10% NaOH solution. 0.10 g of triamcinolone acetonide dissolved in 3 ml of Transcutol was added to the above polymer solution with vigorous stirring and water was added to make a final volume of 100 ml.

2.3. Animal experiments and drug application using phonophoresis

2.3.1. Method of trauma

Male Sprague–Dawley rats (270–300 g) were purchased from the Daehan Laboratory Animal Research Co. (Daejon, Korea), and were given free access to normal standard chow diet (Jail Chow, Korea) and tap water throughout the experiment, the animals were housed, individually, in laminar flow cages maintained at $22 \pm 2\,^{\circ}C$, 50–60% relative humidity, under a 12-h light:12-h dark cycle. The animals were kept in these facilities for at least one week prior to the experiment. Sprague–Dawley rats were fasted for at least 24 h prior to the experiments and were given water freely.

This experiment was carried out in accordance with the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology (USA) in July 1989 and revised in March 1999. The Animal Care Committee in this institution (Chonnam National University) approved this study.

The left leg to thigh of rats anesthetized by ketamine HCI was fixed to a table. An injury was induced by dropping a 200 g iron sinker to the thigh part through an 80-cm tube. At the impacted injury site, the impacted part was wrapped with gauze to prevent the outflow of fluid from the damaged skin (Mendel et al., 1992). Blood was taken from the test groups and compared with that taken from the control group and analyzed histologically.

2.3.2. Drug application using phonophoresis

This study examined the anti-inflammatory effect of TA on the inflammatory lesions of the soft tissue using ultrasound, as a skin absorption method for TA from the gel.

The rats were divided into four groups of six each: after injury, the test groups received different treatments. Group I (control group, no ultrasound treatment) did not receive any treatment. The commercial ultrasound gel for ultrasound was used in the group II (ultrasound group, commercial ultrasound gel without TA with phonophoresis), 0.1% TA gel was used in the group III (0.1% TA gel group with no phonophoresis) and the group IV (0.1% TA gel group with phonophoresis) (Cameron and Monroe, 1992; Wilson et al., 1998). The test gels were applied to the test groups three times, except for the group I, at 0, 24, and 48 h after the trauma.

The rats were divided into four groups of six each: after injury, the test groups received different treatments. Group I (control group, no ultrasound treatment) did not receive any treatment. The commercial gel for ultrasound was used in the group II (ultrasound group, commercial ultrasound gel without TA with phonophoresis), 0.1% TA gel was used in the group III (0.1% TA gel group with no phonophoresis) and the group IV (0.1% TA gel group with phonophoresis) (Cameron and Monroe, 1992; Wilson et al., 1998). The test gels were applied to the test groups three times, except for group I at 0, 24, and 48 h after the trauma.

In the groups II and IV, an ultrasound transducer was used with a technical specification of 1.4 cm^2 of the geometric area (GA), 0.8 cm^2 of the effective radiation area (ERA), and the 6.0 max of the beam nonuniformity ratio (BNR) which means the relationship between the spatial peak intensity and spatial average intensity (Bare et al., 1996). The treatment parameter was a continuous mode, its frequency was 1 MHz, the treatment intensity was 1.0 W/cm^2, the treatment area was four times larger than the ERA, and the treatment time was 5 min.

In the group II, approximately 500 mg of the gel (commercial gel products for ultrasound) was used, and in the group IV, 500 mg of TA gel was applied to the injured part. Pressure was then applied to the ultrasound transducer but without inducing a withdrawal response. The transducer was then placed at a right angle with the surface of the skin in order to reduce the level of energy loss in the ultrasound beam by reflection and refraction.

The application of the transducer was performed using a stroking technique, which had been mainly

used in phonophoresis as a continuous mode, and was rubbed regularly with round movement at 1 in./s of the regulation speed in order to make an equal radiation to the whole part. In the group III, approximately 500 mg of the TA gel was applied using cotton stick and was rubbed lightly five times.

2.4. Analysis of the serum CPK

The blood test was performed by analyzing the CPK, serum enzyme levels. Five milliliters blood samples were taken from each group by the heart puncture method, at 0, 24, and 48 h after the injury. The serum was separated from the collected blood by centrifuging for 15 min at 3000 rpm, and the CPK were analyzed. The serum CPK activity was analyzed using a UV-kinetic method (Burtist, 1994).

2.5. Gross finding

Hemorrhage and hyperemia of the injured part was assessed by a visual inspection. A negative response was noted in those case not showing hemorrhage and hyperemia on the injured part. A positive response was classified into three levels of slight to mild, moderate, and severe, according to the degree of the hemorrhage and hyperemia of the injured parts (Atkins et al., 1991).

2.6. Histological finding

For the histological findings, blood samples were obtained 0, 24, 48, and 72 h after the injury, and the left-injured thigh's skin of rat was then removed and the biceps femoris, connective tissues around muscles, and fat tissues were extracted at size of 1.5 cm × 1.5 cm. The muscle sample was fixed in neutral buffered formalin embedded in paraffin, and sectioned to a 4 μm thickness. The sections were stained with hematoxylin and eosin, and observed using optical microscopy.

Pathologically histological findings in those cases not showing hemorrhage and hyperemia of the capillary vessels and not showing infiltration of inflammatory cells were classified as being a negative response. A positive response was classified into three levels, slight to mild, moderate, severe, according to the degree of hemorrhage and hyperemia of the capillary vessels, and the level of inflammatory cell infiltration.

2.7. Statistical analysis

One-way analysis of variance (ANOVA), using the SPSS 10.0 software in a windows environment was used to determine any significant difference. The differences were considered to be significant at $p < 0.05$. All the means were shown with their standard error (mean ± S.E.).

3. Results and discussion

3.1. Effect of phonophoretic treatment

In a previous paper (Yang et al., 2005), the ultrasound application condition showing the highest permeation was established using an ultrasound frequency (1.0, 3.0 MHz), intensity (1.0, 2.5 W/cm^2), and treatment method (continuous, pulse) with a 0.5% TA gel. The ultrasound application condition showing the highest permeation was a frequency of 1.0 MHz, intensity of 2.5 W/cm^2, and a continuous output (Yang et al., 2005).

This study was carried out to determine the feasibility of using gel formulations for the transdermal delivery of TA in conjunction with phonophoresis, and to develop the carbopol gels of TA. In order to confirm the enhanced percutaneous absorption of TA from the gel formulations following the adoption of ultrasound, the anti-inflammatory effects from the gel containing the TA were evaluated by examining the in vivo change in the serum CPK. The gross and histological findings of the skin were also investigated.

3.2. Effect of phonophoretic treatment on serum CPK

Fig. 1 shows the variation in CPK (l U/l) in the plasma components after the ultrasound treatment in conjunction with the TA gel on a muscle-injured rat. In the group IV, the CPK activity was 351.1 ± 41.5 (l U/l), 405.5 ± 40.0 (l U/l), 323.0 ± 46.9 (l U/l) at 24, 48, and 72 h, respectively. In addition, the CPK activity in groups II and IV was different at 24 and 72 h.

This study showed the highest rate after 48 h, which reduced thereafter. When the TA gel is applied to the skin in conjunction with ultrasound, the CPK activ-

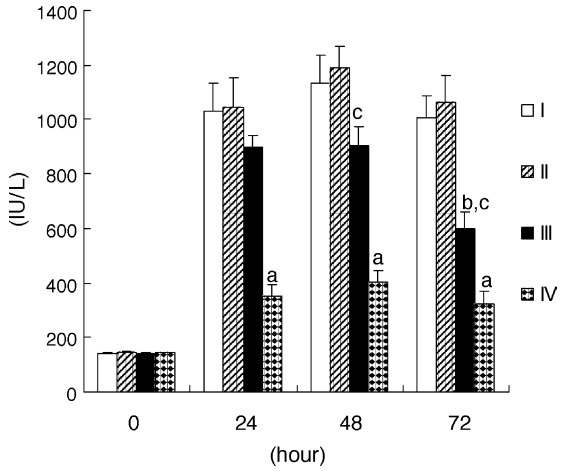

Fig. 1. Variation of serum CPK following adoption of phonophoresis with triamcinolone acetonide gel on muscle-injured rat. (a) Significantly different from groups I to III, (b) significantly different from group I and (c) significantly different from group II.

ity reduced considerably, which is in contrast to the control group. In delayed onset muscle soreness syndrome, the activation of serum CPK increased, which there was a relationship between the level of muscle soreness and CPK activation (Anderson et al., 1984). In addition, the results indicate that the level of CPK activation increased in the exercise-induced muscle damage and ischemia–reperfusion injury (Chanoit et al., 2001; Stupka and Tiidus, 2001).

3.3. Effect of phonophoretic treatment on the gross and histological changes

3.3.1. Change of gross findings

In each group, no hemorrhage or hyperemia was observed in the injured parts of all the animals at time zero (Table 1). In the group II, in 24 h after the injury, severe or moderate hemorrhage and hyperemia were observed in all rats. After 72 h, most rats showed the moderate or mild hemorrhage and hyperemia. In the group III, 24 h after the injury, moderate or mild hemorrhage and hyperemia were shown in most rats. After 72 h, most rats did not show any hemorrhage and hyperemia. In the group IV, 24 h after the injury, moderate or mild hemorrhage and hyperemia were shown in most rats. In 72 h, all rats did not show any hemorrhage and hyperemia.

Table 1
Incidence of gross muscle lesions in rat per each group

Group	Time (h)	Macro			
		–	+	++	+++
I	0	0	0	0	6/6(100)
	24	0	0	2/6(33)	4/6(67)
	48	0	1/6(17)	1/6(17)	4/6(67)
	72	1/6(17)[a]	3/6(50)	2/6(33)	0
II	0	0	0	0	6/6(100)
	24	0	0	3/6(50)	3/6(50)
	48	0	1/6(17)	2/6(33)	3/6(50)
	72	1/6(17)	2/6(33)	3/6(50)	0
III	0	0	0	0	6/6(100)
	24	0	2/6(33)	2/6(33)	2/6(33)
	48	2/6(33)	2/6(33)	2/6(33)	0
	72	3/6(50)	2/6(33)	1/6(17)	0
IV	0	0	0	0	6/6(100)
	24	2/6(33)	2/6(33)	2/6(33)	0
	48	4/6(67)	2/6(33)	0	0
	72	5/6(83)	1/6(17)	0	0

–, no hemorrhage and hyperemia; +, mild hemorrhage and hyperemia; ++, moderate hemorrhage and hyperemia; +++, severe hemorrhage and hyperemia.

[a] Number of rat having lesions/number of rats (%).

During the healing process, tissue injury involving an inflammation reaction is a necessary phenomenon. In the early inflammatory period, hyperemia of the blood vessels, hemorrhage, the increase in exudation, and the appearance of inflammatory cells were observed. Before the recovery period, the appearance of chronic inflammatory cells, an increase in the number of fibroblast cells, the regeneration of the capillary vessels, and the formation of collagen fibers were observed. The permeation of inflammatory cells was not observed in a normal muscle, but the local permeation of inflammatory cells was observed in the injured muscle.

The gross findings from groups I and II, 24 h after the injury, indicated that hemorrhage and hyperemia had a more than moderate score in the injured parts of all the animals. The histological findings showed that hemorrhage and hyperemia of the capillary vessels had a more than moderate score, and the permeation of neutrophilic leukocyte increased among the inflammatory cells. Even 48 h after the injury, the gross findings showed that hemorrhage and hyperemia were present in all the animals at a more than moderate level.

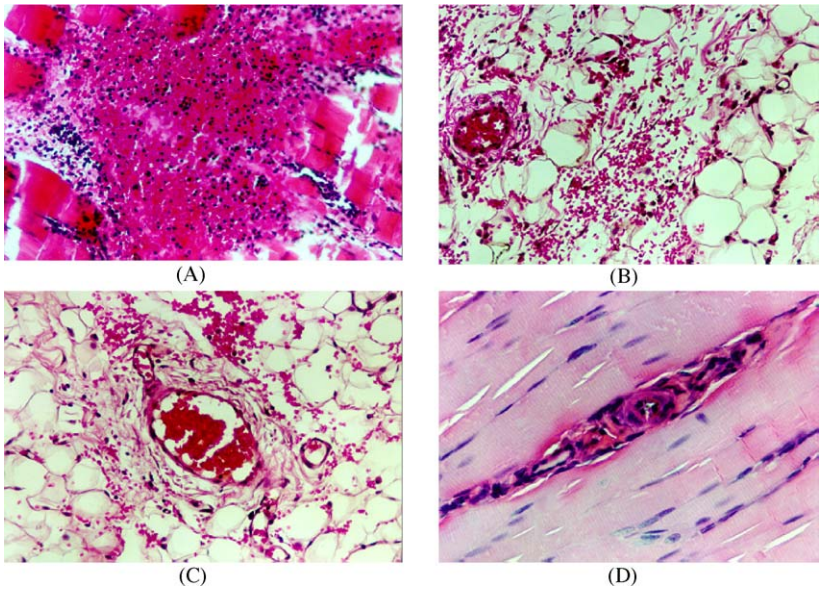

Fig. 2. Histological examinations of all experimental groups at 72 h (hematoxylin and eosin stain ×200). (A) Group I, (B) group II, (C) group III and (D) group IV.

3.3.2. Change of histological findings

In each test group, at 0 h, hemorrhage and hyperemia were not observed in the capillary vessels of the injured parts in any of the animals. In the group I, in 24 h after the injury, severe infiltration of neutrophilic leukocyte and hemorrhage and hyperemia of capillary vessels were observed in all rats. After 72 h, most rats showed the severe or moderate infiltration of neutrophilic leukocyte and hemorrhage and hyperemia (Fig. 2A). In the group II, 24 h after the injury, severe infiltration of neutrophilic leukocyte and hemorrhage and hyperemia of capillary vessels were observed in most rats. 72 h after the injury, diffuse moderate infiltration of inflammatory cells and hemorrhage, and hyperemia of the capillary vessels were observed in most rats. In the inflammatory cells, the infiltration of neutrophilic leukocytes decreased and lymphocytes and monocyte infiltration was observed (Fig. 2B).

In the group III, 24 h after the injury, severe or moderate infiltration of neutrophilic leukocyte and hemorrhage, and hyperemia of capillary vessels were observed in most rats. 72 h after the injury, diffuse slightly infiltration of inflammatory cells and hemorrhage and hyperemia of the capillary vessels. In the inflammatory cells, the infiltration of neutrophilic leukocyte was observed, but it was lower than the groups I and II (Fig. 2C). In the group IV, 24 h after the injury, moderate or mild infiltration of neutrophilic leukocyte and hemorrhage, and hyperemia of capillary vessels were observed in most rats. 72 h after the injury, the focal infiltration of neutrophilic leukocytes was barely observed (Fig. 2D).

The histological findings, 24 h after the injury, showed that hemorrhage and hyperemia as well as the inflammation reaction of the injured part were lower in the groups III and IV in which the TA gel had been applied, than in the groups I and II. The reduction was quite noticeable in the group IV. 48 h after the injury, the level of hemorrhage, hyperemia, and the inflammation reaction were much lower in the groups III and IV than in the groups I and II. Most rats in the group IV showed a negative response showing significant difference from the group III. In the gross and histological findings, 72 h after the injury, most rats in group IV showed a negative reaction.

4. Conclusion

In this study, the anti-inflammatory effects from a gel containing TA were evaluated by examining the in

vivo change in the plasma components. The gross and histological findings on the skin were also investigated.

(1) After the muscle injury, the activity of serum CPKs decreased significantly in the group IV compared with the groups I and II. The variation in the serum CPK activity confirmed the beneficial effect of the TA gel treatment in conjunction with ultrasound.
(2) The gross findings showed that after muscle injury, the group IV moderated the injury more rapidly than the other three groups. The histological finding indicated that the inflammation in the group IV had been relieved within 72 h. These effects were superior to those observed in the other three groups.
(3) These results show that the TA gel using phonophoresis can be developed as a new transdermal delivery technique providing enhanced anti-inflammatory effects.

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