Efficacy of epicutaneous Diractin® (ketoprofen in Transfersome® gel) for the treatment of pain related to eccentric muscle contractions

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Objective: To investigate the effect of epicutaneously applied Diractin® (ketoprofen in Transfersome® gel) on pain induced by eccentric muscle contractions.

Methods: Three pilot studies which were subsequently pooled for a meta-analysis compared the efficacy of a single application of 25 mg ketoprofen in Diractin® to 25 mg oral ketoprofen and placebo for the treatment of pain induced by 50 eccentric contractions of the elbow flexor muscles. In addition, the effect of multiple usage of up to 100 mg ketoprofen in Diractin® bid over seven days on pain induced by walking down stairs with a total altitude of 200 meters was investigated.

Results: A single dose of 25 mg ketoprofen in Diractin® after the elbow flexion exercise was significantly superior to placebo from 5 to 12 hours after treatment and also to oral ketoprofen at some time points after treatment. In contrast, oral ketoprofen was not different to placebo at any time after treatment. Multiple doses of up to 100 mg ketoprofen Diractin® provided significant more pain relief than placebo on muscle pain induced by walking down stairs.

Conclusions: Eccentric exercise-induced muscle soreness was shown to be an appropriate acute pain model to evaluate the efficacy of nonsteroidal anti-inflammatory drugs applied epicutaneously with Transfersome® carriers. Diractin® proved to be efficacious in relieving pain from eccentric muscle contractions and muscle overexercise, respectively. The effect needs to be confirmed in a larger prospective clinical trial.

Keywords: ketoprofen, Transfersome®, epicutaneous application, eccentric muscle contraction, delayed onset muscle soreness

Introduction

Ketoprofen is a peripherally acting nonsteroidal anti-inflammatory drug (NSAID) belonging to the group of substituted 2-phenylpropionic acids.1 NSAIDs have the potential to cause gastrointestinal (GI) side effects such as GI bleeding in a dose-related manner. One approach to reducing these side effects has been to apply NSAIDs to the skin overlying affected joints and muscles.2–5

Diractin® (ketoprofen in Transfersome® gel) is a new, carrier-based formulation for local application that has shown to reduce pain comparable to oral celecoxib in patients with knee osteoarthritis.6 Transfersome® carriers are ultra-deformable lipid vesicles loaded with an active substance and applied epicutaneously in an aqueous preparation. Once the Transfersome® carriers are on the skin, water from the preparation starts to evaporate and deprives carriers of their suspending medium. Transfersome® vesicles reaching their solubility limit are attracted by the higher water content in the skin, resulting in spontaneous migration of the drug-loaded carriers through the skin.
skin barrier. The cutaneous microcirculation cannot clear Transfersome® carriers due to their large size, resulting in high drug concentrations in local tissue with low systemic drug exposure.

Diractin® showed substantially higher drug concentration in target tissues such as muscle as compared to conventional topical products and oral ketoprofen, respectively, in muscle biopsy studies conducted in pigs.

Eccentric exercise causes delayed-onset muscle soreness (DOMS) with pain peaking 24 to 48 hours after exercise. An acute inflammatory reaction is considered one of the underlying mechanisms of DOMS. The muscle strain injury caused by eccentric contractions is characterized morphologically by microscopic damage to the muscle fibers (microtrauma). Neutrophils and tissue macrophages migrate to the damaged muscle tissue, clean up the debris of broken proteins, and then initiate the regeneration phase. Besides a classical inflammatory response, other mechanisms of muscle adaptation and immunological responses in the muscle as well as epimysium might contribute to exercise-induced pain. The use of NSAIDs seems a reasonable therapeutic approach to treat muscle pain induced by eccentric contractions.

This paper reports results from studies investigating the effect of epicutaneously applied Diractin® on pain induced by eccentric muscle contractions after single and multiple applications.

**Methods**

**Single dose application**

The results of three pilot single-center, single-dose, randomized, double-blind, double-dummy, parallel-group, placebo- and active-controlled studies using the model of exercise-induced muscle pain were pooled for a meta-analysis as the individual studies were underpowered to compare efficacy and safety of Diractin® with placebo and oral ketoprofen. All three pilot studies used the same study design, equipment, and application instructions. Studies were performed in accordance with the Declaration of Helsinki and the International Conference on Harmonization of Good Clinical Practice. Study protocols were approved by the relevant institutional review boards.

Subjects provided written informed consent before any study-specific activity was performed. Two hundred ninety-five healthy men and women aged 18 to 45 years were enrolled in the studies. They had to refrain from any strenuous or new physical activities and comply with the respective study protocol while participating in the studies. Moreover, subjects had to be naïve to the exercise so that they could not have resistance trained or performed work that required similar exercise for six months before enrolling in the study. Exclusion criteria focused on avoiding any untoward risk for the study participants and potential interference with the study objectives. Study procedures were performed at four visits. At Visit 1, subjects’ eligibility was assessed by medical history and physical examination. Studies used comparable inclusion/exclusion criteria. Within two weeks after Visit 1, subjects completed the exercise regimen (Visit 2) consisting of 50 consecutive maximal eccentric contractions of the elbow flexor muscles of the nondominant arm. For exercise, subjects were seated on a modified preacher’s bench with the nondominant upper arm resting on a padded support, the wrist fixed between two padded rollers of the exercise lever and the forearm in a fully flexed position. For each eccentric action, the investigator staff had pulled down on the lever, forcing the subjects forearm into a fully extended position as the subjects exerts maximum resistance. Subjects reporting pre-exercise soreness/pain of ≥10 mm on a visual analogue scale (VAS) at Visit 2 were excluded from the study. The Visit 2 exercise was performed in the evening. Approximately 32 hours later, at Visit 3, following an overnight fast, subjects meeting all continuation criteria and reporting at least 50 mm VAS post-exercise soreness/pain received either topical or oral ketoprofen (25 mg each) and oral or topical placebo, respectively. Subjects were fed a standardized meal and remained at the study center for the entire observation period. Subjects rated their muscle soreness/pain intensity prior to performing the exercise, immediately prior to treatment, and at hourly intervals for 12 hours after treatment. At the end of these assessments, subjects provided an overall rating of the study medication. At the final visit (Visit 4), approximately four days after Visit 3, safety and tolerability of the treatments were assessed.

No primary efficacy parameters were specified in the protocols due to the exploratory nature of the studies. Muscle soreness/pain intensity was assessed using VAS and categorical scale (CAT) by completing two actions of elbow flexion and relaxation while holding a 2-lb dumbbell (approximately 0.9 kg). Results for categorical pain intensity difference (CATPID) and visual analogue scale pain intensity difference (VASPID) from baseline (immediately prior to treatment at Visit 3) to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 hours after treatment were determined in the same way in all three studies. The 100 mm horizontal VAS line was labeled “no soreness/pain” on the left (corresponding to “0”) and “very, very sore/painful” on the right (corresponding to “100”).
Categorical ratings of muscle soreness/pain were obtained on a 10-point ordinal rating scale from 0 = none to 9 = extremely intense.

The meta-analysis was performed for the intent-to-treat populations, ie, all subjects that received study medication. The overall statistics were obtained from a meta-analysis of CATPID and VASPID scores with treatment and baseline pain as fixed effects, and study as a random effect. Each study was allowed an individual variance component. Homogeneity in mean responses of the studies was assessed by testing the significance of study variance component in the random effect model. In matters of least-squares (LS) means comparison, the interaction terms drug-by-study and drug-by-baseline were not significant (p > 0.05), and subsequently dropped from the final model. P-values are obtained from testing the equality of all three groups. LS means and standard errors are obtained from an analysis of covariance model with treatment and baseline pain as factors. The differences are that of LS means. P-values are unadjusted, and obtained from testing the equality of two groups.

Multiple dose application

Nineteen subjects were included in this pilot study to investigate the efficacy of multiple dosages of Diractin® (bid over seven days) for the treatment of exercise-induced muscle pain. To induce muscle pain, the subjects walked down stairs using twelve consecutive runs of 18 m difference in total altitude per run (120 stairs with each stair 15 cm high). This corresponds to a total altitude of approximately 200 m.

Eligible subjects came in at the day after exercise and were randomly allocated to two subgroups which received 100 mg ketoprofen in Diractin® bid either on the left or right target muscle (thigh). The subjects were asked to apply the product open (nonocclusively) over the entire surface of either the left or the right thigh. The contralateral leg was used as (untreated) control. The primary target area for all subjects was the thigh. If in some subjects the calf became symptomatic, Diractin® was also administered epicutanously to the entire surface of the left or right calf. Again, the contralateral calf was used as untreated control. Categorical ratings of muscle soreness/pain were recorded on a 10-point ordinal rating scale (0 = none to 9 = extremely) twice daily in a diary, immediately before study drug application. For statistical testing, the sum of categorical pain scores for the treated muscle were compared to the untreated control muscle using the Wilcoxon test. Nonparametric testing was chosen due to the small sample size and since it was assumed prospectively that normal distribution of data is not guaranteed.

### Results

#### Single dose application

The demographic data between the three treatment groups was comparable with an age range of 18 to 45 and the majority of subjects being males (Table 1). Also baseline pain was comparable between the treatment groups with CAT scores around 6.4 and VAS scores between 70.5 and 73.7 mm (Table 2).

The estimated hourly VASPID and CATPID results from one to 12 hours after treatment for Diractin® (IDEA-033) versus placebo are presented in Figures 1a and 1b, for Diractin® (IDEA-033) versus oral ketoprofen in Figures 2a and 2b, and for oral ketoprofen versus placebo in Figures 3a and 3b. Mean VASPID for 25 mg ketoprofen in Diractin® versus placebo increased over time reaching a plateau of significant differences between Diractin® and placebo from five to 12 hours after treatment (Figure 1a). Similar results were determined for mean CATPID (Figure 1b). Mean VASPID for 25 mg ketoprofen in Diractin® versus oral ketoprofen were significantly different only at 12 hours after treatment (Figure 2a), whereas mean CATPID showed significant difference between Diractin® and oral ketoprofen from seven to 12 hours after treatment (Figure 2b). No significant differences in mean VASPID and CATPID between oral ketoprofen and placebo were determined at any time after treatment (Figures 3a and 3b).

#### Multiple dose application

Nineteen subjects were included (Table 1). The calf was treated in nine subjects. The subjects documented higher pain scores for the calf than for the thigh, maximum pain was observed within 24 to 48 hours after exercise.

### Table 1 Demographics of study populations

| Variable                      | Placebo | Diractin® | Oral ketoprofen |
|-------------------------------|---------|-----------|-----------------|
| Single dose study             |         |           |                 |
| Number of subjects            | 88      | 118       | 89              |
| Age                           | 26.3 (18–45) | 26.6 (18–44) | 26.4 (18–45)   |
| Sex                           | 34/54   | 51/67     | 33/56           |
| Multiple Dose Study Number of subjects | 19      |           |                 |
| Age                           | 23.4 (18–44) |           |                 |
| Sex                           | 10/9    |           |                 |
(average categorical pain score at time of maximum pain for untreated thigh: 2.6 ± 2.2; for untreated calf: 5.1 ± 1.9). The average sum of categorical pain scores documented for the treated thighs (n = 19) was 12.4 (range 0 to 29) and 14.8 (range 0 to 42) in the untreated thighs. The sum of pain scores was significantly lower in the treated thighs than in the untreated thighs (Wilcoxon p-value = 0.039; Table 3). For the calf (n = 9), the average sum of pain scores documented for the treated calves was 29.4 (range 13 to 55) and in the untreated 32.9 (range 6 to 65). The difference between treated and untreated areas was not statistically significant (Wilcoxon p-value = 0.359) probably due to the small sample size.

**Discussion and conclusions**

Different pain models have been developed for evaluating the efficacy of topical and oral NSAIDs. Besides defined pain situations in patients, eg, after surgery or tooth extraction, additional models were explored that would be more specific to muscular pain.

Experimental pain resulting from continuous pressure infusion of phosphate-buffered low pH solution (pH 5.2) into the belly of the radial flexor carpi muscle induced a localized dull-aching or stinging muscle pain sensation. However, it remained unclear whether this experimental induction of muscle pain was predictive for actual muscle pain. Therefore, the induction of muscle soreness/pain by controlled, standardized eccentric contractions producing DOMS was explored. DOMS is defined as skeletal muscle discomfort that peaks 24 to 48 hours after exercise. At that time, a significant increase in prostaglandin E was observed, suggesting that acute inflammation is one of the underlying mechanisms of DOMS. More recent studies indicate that a classical inflammatory reaction might not be the main reason

| Table 2 Mean pain scores before treatment for single dose application studies and the corresponding 95% confidence intervals of difference (CI) between treatment groups |
|---------------------------------|----------------|----------------|-----------------|-----------------|
| Variable                        | Placebo        | Diractin®      | Oral ketoprofen | CI Plac. vs Diractin® | CI Plac. vs Oral | CI Diractin® vs Oral |
| Categorical pain scale          | 6.36           | 6.54           | 6.41            | (-0.12; 0.49)        | (-0.27; 0.36)    | (-0.16; 0.44)       |
| Visual analog scale (mm)        | 70.6           | 73.7           | 72.3            | (-0.67; 7.00)        | (-2.32; 5.80)    | (-2.40; 5.25)       |
Figure 2a Estimated mean hourly visual analogue scale pain differences (VASPiD) between 25 mg ketoprofen in Diractin® and 25 mg oral ketoprofen from 1–12 hours after treatment (mean differences and 95% CI).
Abbreviation: CI, confidence interval.

Figure 2b Estimated hourly categorical pain intensity differences (cATPiD) between 25 mg ketoprofen in Diractin® and 25 mg oral ketoprofen from 1–12 hours after treatment (mean differences and 95% CI).
Abbreviation: CI, confidence interval.

Figure 3a Estimated mean hourly visual analogue scale pain differences (VASPiD) between 25 mg oral ketoprofen and placebo from 1–12 hours after treatment (mean differences and 95% CI).
Abbreviation: CI, confidence interval.

Figure 3b Estimated hourly categorical pain intensity differences (CATPiD) between 25 mg oral ketoprofen and placebo from 1–12 hours after treatment (mean differences and 95% CI).
Abbreviation: CI, confidence interval.
for DOMS, but other mechanisms of muscle adaptation and changes in immunological parameters in the epimysium might contribute to this process.\textsuperscript{14} The controversy between several studies might also be related to the effects of muscle biopsies itself.\textsuperscript{14}

The effect of NSAIDs on muscle pain induced by eccentric contractions is still controversial. A lack of efficacy of oral NSAID treatment was reported for flurbiprofen and ibuprofen.\textsuperscript{16,17} The influence of flurbiprofen on subjective soreness was investigated in six healthy young men.\textsuperscript{16} The subjects performed one concentric and two eccentric work bouts of 30 min at 80\% of the individual maximal work load on the bicycle ergometer. No influence of 50 mg flurbiprofen tid on subjective soreness was reported. The influence of 400 mg ibuprofen tid on active range of motion, perceived pain, and peak concentric torque measurements of the nondominant arm was assessed in 40 subjects in a randomized, double-blinded, repeated measures design.\textsuperscript{17} Subjects performed an eccentric exercise protocol of the elbow flexors. No differences among treatments were observed for any of the dependent variables at any time up to 96 hours after the exercise.

In contrast, a randomized, double-blind, placebo-controlled crossover study of 200 mg oral naproxen tid for 10 days indicated superiority of naproxen to placebo in improving objective recovery as per magnetic resonance imaging results and effects on thigh soreness in particular during the first four days after exercise.\textsuperscript{18} Subjects performed 10 sets of 7–10 eccentric actions with each quadriceps femoris muscle with a load equal to 85\% of the eccentric one repetition maximum. Study drug treatment began immediately after exercise. In a randomized, double-blind, placebo-controlled parallel-group study, the influence of 100 mg ketoprofen in a pluronic lecithin organogel applied tid on DOMS was investigated in 32 young healthy men.\textsuperscript{19} Subjects performed a leg extension and flexion exercise program designed to create DOMS in quadriceps muscles. Subjects were randomly assigned to receive any combination of topical ketoprofen or placebo gel to their right and left quadriceps immediately following the exercise. Within-subjects analysis (n = 16) showed a significant reduction in DOMS scores in legs receiving topical ketoprofen compared with legs receiving placebo (P = 0.002 at 48 hours, and P < 0.001 at 24 and 48 hours combined). Between-subjects analysis (n = 16) showed a marginally significant reduction in DOMS scores at 48 hours (P = 0.05 in right legs and P = 0.053 in left legs).

The studies published varied not only with respect of the NSAID investigated, but also whether study drug was applied immediately after exercise or at peak of the soreness. We therefore decided to explore the various options in different settings, eg, single and multiple dosing, using oral ketoprofen as an active comparator, or investigating the therapeutic effect of Diractin\textsuperscript{®} at different muscular regions, ie, elbow flexor muscles or thigh and calf muscles, respectively.

A single dose of 25 mg ketoprofen in Diractin\textsuperscript{®} was significantly superior to placebo in treating muscle pain induced by eccentric contractions and as expressed by mean VASPID and CATPID from five to 12 hours after treatment and also to 25 mg oral ketoprofen at some time points after treatment. In contrast, a single dose of 25 mg oral ketoprofen was statistically not different to placebo at any time after treatment. Diractin\textsuperscript{®} application was also effective as compared to untreated controls after multiple applications. The appropriate dose and treatment regimen still needs to be confirmed in a larger randomized, controlled, prospective clinical trial.

DOMS was shown to be an appropriate pain model for evaluating the therapeutic effect of epicutaneous Diractin\textsuperscript{®} on acute muscle pain. The results indicate that Diractin\textsuperscript{®} appears to be a valuable option for treating musculoskeletal pain.

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**References**

1. Veys EM. 20 years’ experience with ketoprofen. Scand J Rheumatol Suppl. 1991;90:1–44.
2. Matucci-Cerinic M, Casini A. Ketoprofen vs etofenamate in a controlled double-blind study: evidence of topical effectiveness in soft tissue rheumatic pain. Int J Clin Pharmacol Res. 1988;8:157–160.

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**Table 3** Sum of categorical pain intensity differences after multiple use

| Muscle | Target area | N   | Mean | SD  | Median (Range) | P – value (Wilcoxon) |
|--------|-------------|-----|------|-----|----------------|---------------------|
| Thigh  | Treated     | 19  | 12.4 | 10.3| 13 (0–29)      | 0.039               |
|        | Not treated | 19  | 14.8 | 13.1| 12 (0–42)      |                     |
|        | Treated     | 9   | 29.4 | 14.9| 26 (13–55)     | 0.359               |
|        | Not treated | 9   | 32.9 | 18.5| 35 (6–65)      |                     |
3. Baixauli F, Inglez F, Alcantara P, Navarrete R, Puchol E, Vidal F. Percutaneous treatment of acute soft tissue lesions with naproxen gel and ketoprofen gel. *J Int Med Res.* 1990;18:372–378.

4. Airaksinen O, Venalainen J, Pietilainen T. Ketoprofen 2.5% gel versus placebo gel in the treatment of acute soft tissue injuries. *Int J Clin Pharmacol Ther Toxicol.* 1993;31:561–563.

5. Waikakul S, Penkitti P, Soparat K, Boonsanong W. Topical analgesics for knee arthrosis: a parallel study of ketoprofen gel and diclofenac gel. *J Med Assoc Thai.* 1997;80:593–597.

6. Rother M, Lavins BJ, Kneer W, Lehnhardt K, Seidel EJ, Mazgareanu S. Efficacy and safety of epicutaneous ketoprofen in Transfersome® (IDEA-033) and Placebo in osteoarthritis of the knee: Multicentre randomized controlled trial. *Ann Rheum Dis.* 2007;66:1178–1183.

7. Ceve G, Schatzlein A, Richardsen H. Ultradeformable lipid vesicles can penetrate the skin and other semi-permeable barriers unfragmented. Evidence from double label CLSM experiments and direct size measurements. *Biochim Biophys Acta.* 2002;1564:21–30.

8. Ceve G, Mazgareanu S, Rother M. Preclinical characterisation of NSAIDs in ultradeformable carriers or conventional topical gels. *Int J Pharm.* 2008;360(1–2):29–39.

9. Clarkson PM, Hubal MJ. Exercise-induced muscle damage in humans. *Am J Phys Med Rehabil.* 2002;81:S52–S69.

10. MacIntyre DL, Reid WD, McKenzie DC. Delayed muscle soreness. The inflammatory response to muscle injury and its clinical implications. *Sports Med.* 1995;20:24–40.

11. Miles MP, Clarkson PM. Exercise-induced muscle pain, soreness, and cramps. *J Sports Med Phys Fitness.* 1994;34:203–216.

12. Smith LL. Acute inflammation: the underlying mechanism in delayed onset muscle soreness? *Med Sci Sports Exerc.* 1991;23:542–551.

13. Friden J, Sjostrand M, Ekblom B. Myofibrillar damage following intense eccentric exercise in man. *Int J Sports Med.* 1983;4:170–176.

14. Malin C, Sjödin B, Sjöberg B, Lenkei R, Renström P, Lundberg IE, Ekblom B. Leuocytes, cytokines, growth factors and hormones in human skeletal muscle and blood after uphill or downhill running. *J Physiol.* 2004;556(Pt3):983–1000.

15. Steen KH, Wegner H, Meller ST. Analgesic profile of peroral and topical ketoprofen upon low pH-induced muscle pain. *Pain.* 2001;93:23–33.

16. Kuipers H, Keizer HA, Verstappen FT, Costill DL. Influence of a prostaglandin-inhibiting drug on muscle soreness after eccentric work. *Int J Sports Med.* 1985;6:336–339.

17. Stone MB, Merrick MA, Ingersoll CD, Edwards JE. Preliminary comparison of bromelain and Ibuprofen for delayed onset muscle soreness management. *Clin J Sport Med.* 2002;12:373–378.

18. Dudley GA, Czerkawski J, Meinrod A, Gillis G, Baldwin A, Scarpone M. Efficacy of naproxen sodium for exercise-induced dysfunction muscle injury and soreness. *Clin J Sport Med.* 1997;7:3–10.

19. Cannavino CR, Abrams J, Palinkas LA, Saglimbeni A, Bracker MD. Efficacy of transdermal ketoprofen for delayed onset muscle soreness. *Clin J Sport Med.* 2003;13:200–208.