Online Resource 1: Complete inclusion and exclusion criteria

| Inclusion criteria |
|--------------------|
| • Age 6 to 16 years, either gender |
| • Patients aged 6–9 years must have their parent/legally authorized representative complete a Parental Permission Form for informed consent; patients between 10–16 years of age were required to complete a Children’s Assent Form and have their parent/legally authorized representative complete a Parental Permission Form |
| • Minor soft tissue injury <96 hours of study entry that is considered to be clinically significant by the Investigator |
| • Spontaneous pain of greater than or equal to moderate intensity (i.e. pain of ≥3 on the 6-point Wong-Baker Faces scale for pain severity assessment by patients) |
| • Negative urine pregnancy test at screening for female patients of childbearing potential |
| • Ability to read and speak English |
| • Availability for 2 weeks following study enrollment |

| Exclusion criteria |
|--------------------|
| • Major soft tissue injury (fractures were excluded if the injury was stabilized with a device, e.g. a hard cast, that could not be removed to allow a topical system to be applied to the site of injury) |
- Open skin lesion or any dermatological condition (e.g. skin infection, eczema) within the injured area
- Injury is midline or involving the spine, digits, or hands
- Prior injury to the same site <3 months before the study
- Major or minor prior injuries (≥3) to the same region in the past
- Injury occurring >96 hours prior to study entry
- Prior use of topical medication to the involved area (<48 hours before the study entry)
- Concomitant use of drugs which may be susceptible to interactions with diclofenac, or affect its safety if used concomitantly (serotonin-selective reuptake inhibitors, lithium, digoxin, anticoagulants, antidiabetic agents, cyclosporin, methotrexate, quinolone antimicrobials, other NSAIDs, steroids and diuretics)
- Allergic disorders, including asthma or urticaria, but only if associated with exposure to aspirin or an NSAID
- Coagulation defects
- Prior use of OTC analgesics or short-acting NSAIDs (ibuprofen, ketoprofen) <6 hours before study entry (acetaminophen permitted until the time of study entry)
- Prior use of narcotic analgesics <7 days of study entry
- Prior use of systemic anti-inflammatory steroidal drugs by any route of administration, <60 days of study entry
- Prior use of long-acting NSAIDs such as piroxicam or naproxen since injury
- Patients suffering from psychiatric disorders including depression
- Disabled patients, if the disability prevents them from either assessing their pain or safely using the topical system (e.g. pervasive developmental disorders such as autism, Asperger syndrome, Rett syndrome, Heller’s syndrome, severe attention deficit hyperactivity disorders, other severe mental retardation of traumatic,
- History of alcohol or drug use <1 year before the study
- Severe cardiac, renal or hepatic impairment
- Severe systemic diseases (e.g. cancer, severe acute infection)
- Immunocompromised patients (due to underlying disease or medication use)
- Prior history of chronic pain disorder
- Prior history of GI bleeding/ulcers, liver or kidney disease
- Hypersensitivity to diclofenac or other NSAIDs including aspirin
- Female patients who are pregnant or breastfeeding
- Patients participating in or been involved in other clinical investigations <3 months preceding the study

*GI* gastrointestinal, *NSAID* non-steroidal anti-inflammatory drug, *OTC* over the counter
Clinical Drug Investigation

Safety and Efficacy of the FLECTOR (Diclofenac Epolamine) Topical System in Children with Minor Soft Tissue Injuries: A Phase IV Non-Randomized Clinical Trial

Christopher A. Jones, PhD, • Fred K. Hoehler, PhD, • Valeria Frangione, PhD, Gilbert Ledesma, MD, • Paul P. Wisman Jr, MD, • Clarence Jones PhD

Corresponding author:
Valeria Frangione, PhD, IBSA Institut Biochimique SA, Via del Piano, 29, 6915 Pambio-Noranco, Lugano, Switzerland E-mail: Valeria.Frangione@ibsa.ch

Online Resource 2: Analytical Methodology

| 1. Experimental |
|------------------|
| **Study Samples Information:** |
| Storage Temperature: -20°C |
| Number of Samples Analyzed: 205 |
| Longest Duration of Sample Storage (collection date to last extraction date): 224 days, within the validated stability period (701 days) |
| **Analytical Method:** |
| Analyte: Diclofenac |
| Internal Standard: Diclofenac-d₄ |
| Calibration Range: 50 to 50000 pg/mL |
| Biological Matrix: Human EDTA K₂ Plasma |
| Assay Volume Required: 0.100 mL |
| Sample Extraction: Automated liquid-liquid extraction |
| Type of Assay: LC/MS/MS (API 5000) |
| Quantitation Method: Peak area ratio |
| Calibration Regression: Linear |
| Weighting Factor: 1/C² [Peak area ratios (analyte/internal standard) versus the nominal concentration of the calibration standards] |
| Calibration equation: y = mx + b |
| Determination factor: r² |
| **Method Validation Summary:** |
| Calibration Curve Range: 50.00 to 50000.00 pg/mL |
| Linearity: r² ≥ 0.9917 |
| Calibration Curve Accuracy and Precision: %Bias: -3.68 to 2.76, %CV: 3.06 to 6.53 |
| Between-Run Accuracy and Precision: %Bias: -5.15 to 1.73, %CV: 2.81 to 16.69 |
| Within-Run Accuracy and Precision: %Bias: -8.07 to 6.73, %CV: 2.25 to 4.95 |
| Recovery of Analyte: 81.77, 89.10 and 87.01% |
| Recovery of Internal Standard: 80.73% |
| Dilution Integrity: %Bias: -3.56, %CV: 3.39 |
Lower Limit of Quantitation (LLOQ): Signal to noise ratio at 50.00 pg/mL: 12

Freeze and Thaw Stability: 4 cycles at -20°C

Short-Term Stability of Analyte in Matrix: 25h12min at room temperature

Short-Term Stability of Analyte in Solution (High Concentration): 23h51min at room temperature

Short-Term Stability of IS in Solution (High Concentration): 26h00min at room temperature

Short-Term Stability of Analyte and IS in Solution (Low Concentration): 26h05min at room temperature

Long-Term Stability of Analyte in Matrix: 701 days at -20°C

Post-Preparative Stability: 71h08min at room temperature

Long-Term Stability of Analyte in Solution (High Concentration): 960 days at -20°C

Long-Term Stability of Analyte in Solution (Low Concentration): 335 days at -20°C

Long-Term Stability of IS in Solution (High Concentration): 265 days at -20°C

Long-Term Stability of IS in Solution (Low Concentration): 76 days at -20°C

Reference Standards:
Diclofenac (U.S. Pharmacopeia) / Diclofenac-d4 (C/D/N Isotopes)

Preparation of Stock Solutions:
Four separate stock solutions of diclofenac and two stock solutions of the internal standard were prepared.

Two analyte stocks solutions were used to prepare the calibration standards and the others were used to prepare the quality control samples.

Preparation of Calibration Standards and Quality Control Samples:
Calibration standard and quality control working spiking solutions were prepared by diluting the analyte stock solutions. These working spiking solutions were then spiked in blank matrices (Human EDTA K2 Plasma) to obtain, in addition of blank and zero calibrants, eight non-zero calibrants and four levels (150-2500-25000-37500 pg/mL) of quality control samples.

2. Results

Analysis of Study Samples:
Study samples were analyzed once using a duplicate calibration curve and using four sets of quality control samples (low, intermediate, medium and high QCs) analyzed at least in duplicate (to have at least 5% of the number of study samples). A total of 205 study samples were analyzed.

Study Sample Reassays:
There were no reassays in this project.

Incurred Samples Reanalysis:
A total of 52 samples were selected for the incurred sample reproducibility test to demonstrate that results obtained from study sample analysis are reproducible. A total of 100.00% of the reanalyzed were within ± 20% of the original values.

In-Study Method Performance:
Back-calculated concentrations of calibration standards from the accepted analytical runs: %Bias = -1.61 to 1.64, %CV = 1.93 to 7.89.

Within-run and between-run accuracy and precision of quality control samples from the accepted analytical runs: %Bias = -1.11 to 1.90, %CV = 2.01 to 9.42.

No individual reintegraions of chromatograms have been performed in this project.
Clinical Drug Investigation

Safety and Efficacy of the FLECTOR (Diclofenac Epolamine) Topical System in Children with Minor Soft Tissue Injuries: A Phase IV Non-Randomized Clinical Trial

Christopher A. Jones, PhD, • Fred K. Hoehler, PhD, • Valeria Frangione, PhD, Gilbert Ledesma, MD, • Paul P. Wisman Jr, MD, • Clarence Jones PhD

Corresponding author:
Valeria Frangione, PhD, IBSA Institut Biochimique SA, Via del Piano, 29, 6915 Pambio-Noranco, Lugano, Switzerland E-mail: Valeria.Frangione@ibsa.ch

Online Resource 3: Study CRO-PK-01-72

Outline

In CRO-PK-01-72, a single centre, open, randomized, repeated three-way cross-over comparative pharmacokinetic (PK) study, diclofenac bioavailability from the Flector® patch was compared with that of a different diclofenac-containing topical formulation (the development of which was subsequently abandoned due to quality issues) and with oral diclofenac (a 50 mg tablet). Additionally, plasma levels of epolamine (the base of the diclofenac salt present in Flector patch) and of its main human metabolite, N-oxide epolamine, were measured.

Of the 24 healthy male and female subjects who had both Flector® and the other patch applied (4 days, b.i.d) in two subsequent period separated by an adequate wash-out, 12 subjects also had 50 mg diclofenac (Voltaren®, Novartis) administered orally as a single dose (in a third study period).

The main steady state AUC_{24h} for Flector® was compared to the AUC_{∞} for Voltaren® as an expression of the relative bioavailability of diclofenac from the patch; it was found to be 0.7 ± 0.39%. The C_{max} was 1.3 ± 0.6 ng/mL and 1214 ± 750 ng/mL for Flector® patch and Voltaren® tablet respectively. Hence the overall exposure in terms of AUC was a hundred-fold lower and in terms of C_{max} a thousand-fold.

Further, the N-oxide epolamine levels were below lower limit of quantification (LLQ) and lower limit of detection (LLD), 10 and 5 ng/ml, respectively. The amount of absorbed epolamine, approximately calculated, seems less than 2 mg, a value which is also consistent with the availability of the counterion, diclofenac.
Synopsis

**Study CRO-PK-01-72**

**Title of the study:** Comparative bioavailability of a new tape formulation of diclofenac epolamine vs. epicutaneous Tissugel® and oral diclofenac-Na Voltaren® in healthy volunteers

**Investigator(s):** Antonio Rusca, MD, FMH

**Study site:** Cross Research s.a. – Phase I Unit, Via F.A. Giorgioli, CH-6864 - Arzo, Switzerland

**Publication (reference):** not published

**Study Period:** Date of first enrolment: 09Oct02 - Date last volunteer completed: 09Nov02

**Objectives:** To compare the diclofenac bioavailability of a new patch formulation applied o.d. and of Flector Tissugel (Flector patch) applied b.i.d. To assess the relative bioavailability of diclofenac in comparison to the oral systemic form (50 mg Tablet).

**Methods:** Phase I, single centre, open, randomised, three-way cross-over comparative study.

**Number of subjects (planned and analysed):** 24 healthy male and female volunteers

**Criteria for inclusion:**
Males and females of no childbearing potential (using adequate contraceptive measures) and not lactating, 18-45 years old; 18.5-24.9 kg/m²; normal values of BP (100-139 mm Hg SBP and 50-89 mmHg DBP) and of HR (50-90 bpm), measured after 5 min of rest (sitting position); no clinically relevant abnormalities at ECG (12 leads); no clinically relevant abnormal physical findings, no abnormal laboratory values indicative of physical illness; no history of anaphylaxis to drugs or allergic reactions in general, which the Investigator considers may affect the outcome of the study, in particular no history of hypersensitivity to NSAIDs; no relevant history of renal, hepatic, gastrointestinal, cardiovascular, respiratory, skin, haematological, endocrine or neurological diseases, that may interfere with the aim of the study, in particular no history of peptic ulcer, bronchial asthma and/or bronchospasm, urticaria; no skin abnormalities likely to be aggravated by the study product, such as dermatological disease or infection, rash, atrophic, fragile or abnormally dry skin, cuts or abrasions at the application site, sensitisiveness to topical preparations or adhesive dressings. No signs of dermatitis or any ascertained skin disease or abnormality affecting the exposure area and the surroundings. No skin wounds; no medication, including OTC products, during 2 weeks before the start of the study, in particular no use of NSAIDs; no participation in the evaluation of any drug, no blood donations for 3 months prior to this study; no blood donation during the 3 months prior to the study; no history of drug, alcohol (≤2 drinks/day for men and 1 for women, defined according to USDA Dietary Guidelines 2000) or tobacco abuse (>10 cigarettes/day); ability to comprehend the full nature and purpose of the study, including possible risks and side effects; ability to co-operate with the Investigator and to comply with the requirements of the entire study; written informed consent prior to inclusion in the study.

**Test product, dose, mode of administration, batch N°:** DHEP-Tape/04L, containing 130 mg DHEP (100 mg diclofenac Na), 1 tape/day was applied for 4 consecutive days every 24 h, batch 102/IB-43, expiry JAN03

**Duration of treatment:** 1 tape (or patch)/day applied for 4 consecutive days

**Reference therapy, dose, mode of administration, batch N°:**
- Flector EP Tissugel® (i.e. Flector patch), containing 182 mg DHEP (140 mg diclofenac Na), 2 plasters/day applied for 4 consecutive days every 12 h, batch 102/IB-43, MAR05
- Voltaren® tablets, containing 50 mg diclofenac Na, 1 single administration, batch T2039, MAY07

**Criteria for evaluation:** Diclofenac plasma level after application of DHEP-Tape 04/L and Flector EP Tissugel® and administration of Voltaren® tablets. Concentrations of diclofenac in plasma samples were determined at Pharmakin GmbH (Germany) using a validated LC-MS/MS method (LOQ: 0.15 ng/mL plasma).

**Criteria for evaluation (safety):** Local tolerability assessed by the volunteers as pain at peeling off of tape or plaster and by the Investigator as skin reaction, AEs, BP, HR, BW, ECGs, laboratory analysis

**Statistical methods:** PK analysis was performed using Kinetica™ Version 4.0 InnaPhase Corporation, Philadelphia, USA. The data documented in this trial and the clinical parameters measured were described using classic descriptive statistics for quantitative variables and frequencies (or %) for
Results: The bioavailability of diclofenac determined at the steady state, after 4 days of application of a new topical delivery system, DHEP tape, was compared, in a cross-over design, to that obtained with the already marketed Flector EP Tissugel plaster given bid for 4 consecutive days.

The main steady state PK parameters measured or calculated from diclofenac plasma concentration after the two treatments are shown below:

|                  | Flector EP | DHEP tape | Flector EP | DHEP tape | Flector EP | DHEP tape | Flector EP | DHEP tape | PTF (%) |
|------------------|------------|-----------|------------|-----------|------------|-----------|------------|-----------|---------|
| C_{\text{SSmax}} (ng/mL) | 1.73       | 6.62      | 0.94       | 3.77      | 1.27       | 5.14      | 60.41      | 54.92     |         |
| SD               | 0.89       | 2.75      | 0.41       | 1.60      | 0.574      | 2.04      | 17.29      | 15.89     |         |
| CV%              | 51.30      | 0.41      | 43.88      | 0.42      | 45.24      | 0.40      | 28.62      | 0.29      |         |
| Min              | 0.48       | 2.89      | 0.24       | 1.52      | 0.373      | 2.32      | 35.8       | 33.19     |         |
| Max              | 4.17       | 12.77     | 2.10       | 7.86      | 2.809      | 9.40      | 103.14     | 109.36    |         |
| N                | 24         | 24        | 24         | 24        | 24         | 24        | 24         | 24        |         |

Mean diclofenac concentrations obtained after treatment with DHEP-tape are about 4 times higher than the corresponding values obtained with Flector EP Tissugel®. This evidence is confirmed by AUC_{SS} values, whose comparison, after dose normalisation, leads to estimate an average improvement of the relative bioavailability of the new tape of more than 12 times, i.e. 1238.30 ± 408.89%.

Continued below…
In a 3rd study period 12 out of the 24 volunteers, equally divided into males and females, underwent a single administration of diclofenac 50 mg tablets (Voltaren® Novartis). The Main PK parameters of diclofenac after single oral administration of Voltaren® 50 mg tablets were the followings:

|     | N=12 | T1/2 (h) | MRT (h) | Cmax (ng/mL) | Tmax (h) | AUCt (ng*h/mL) | AUC∞ (ng*h/mL) |
|-----|------|----------|---------|--------------|----------|----------------|----------------|
| Mean| 1.93 | 3.24     | 1214.4  | 2.88         | 1754.2   | 1764           | 1059.9         |
| SD  | 0.50 | 1.23     | 750.25  | 1.46         | 1060.7   | 1060.7         | 60.13          |
| CV% | 26.12| 37.91    | 61.78   | 50.91        | 60.42    | 60.42          | 10.13          |
| Min | 1.19 | 0.88     | 142.27  | 0.5          | 606.84   | 614.23         | 614.23         |
| Max | 2.63 | 5.65     | 2920.9  | 6            | 4285.9   | 4291.8         |                 |

Calculated plasma diclofenac AUC∞, taken as reference with the aim of approximately evaluating the relative availability of DHEP-tape and Flector EP Tissugel®, gave the following results:

|                | %Frel DHEP tape vs oral | %Frel Flector EP Tissugel vs oral |
|----------------|-------------------------|----------------------------------|
| Mean           | 4.63                    | 0.70                             |
| SD             | 3.20                    | 0.39                             |
| CV%            | 69.10                   | 56.42                            |
| Min            | 1.49                    | 0.15                             |
| Max            | 10.79                   | 1.45                             |
| N              | 12                      | 12                               |

These results confirm the higher relative bioavailability of diclofenac if released from the new transdermic system of DHEP-tape as compared to Flector EP Tissugel®.

Conclusions:

The overall evidence of the present study demonstrates that the new once daily DHEP-tape allows a higher bioavailability of diclofenac as compared to the already marketed Flector EP Tissugel®, even though this last is applied bid and the dose of each plaster contains 182 mg of diclofenac epolamine instead of the 130 mg of the tape.

The amount of absorbed epolamine, approximately calculated on the basis of previous oral data, seems less than 2 mg, a value which is also consistent with the availability of the counterion diclofenac.

Local tolerability and safety of both DHEP-tape and Flector EP Tissugel® were excellent

Date of the report: Final version, 16FEB09