Heliyon 7 (2021) e06518

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Clinical assessment of the use of topical liquid diclofenac following laser microporation of cutaneous neurofibromas in individuals with neurofibromatosis type 1

Lisa Brauer Oliveira [a,*,1], Mauro Geller [b,c,d,e,2], Karin Soares Cunha [f,g,3], Alessandra Santos [h,1], Allan Bernacchi [i,4], Allan E. Rubenstein [j,5], Sanyu Takirambudde [k,5], Spyros Mezitis [l,m,6], Carolina de Almeida Ito Brum [n,7], Luiz Guilherme Darrigo Jr. [o,8], Marcia Gonçalves Ribeiro [p,q,1]

[a] Postgraduate Program in Clinical Medicine, Universidade Federal do Rio de Janeiro (UFRJ) – Rio de Janeiro, Brazil
[b] Medical Genetics Service, Instituto de Puericultura e Pediatria Martagão Gesteira Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil
[c] Department of Immunology and Microbiology, Centro Universitário Serra dos Órgãos (UNIFESO), Teresópolis, Brazil
[d] Hospital Israelita Albert Einstein, São Paulo, Brazil
[e] Department of Clinical Immunology, Instituto de Pós-Graduação Médica Carlos Chagas – Rio de Janeiro, Brazil
[f] Postgraduate Program in Pathology, Universidade Federal Fluminense (UFF) Medical School, Niterói, Brazil
[g] Pathology Department, Faculdade de Medicina - Universidade Federal Fluminense (UFF), Niterói, Brazil
[h] Postgraduate Program in Clinical Medicine, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil
[i] Department of Plastic Surgery, Serviço Prof. Pitanguy, Santa Casa da Misericórdia do Rio de Janeiro, Rio de Janeiro, Brazil
[j] Department of Neurology and Pediatrics, NYU Langone Medical Center, New York, NY, USA
[k] Department of Pediatrics, New York University Langone Medical Center, New York, NY, USA
[l] Department of Endocrinology/Clinical Medicine, Weill Medical College of Cornell University, New York, NY, USA
[m] New York Presbyterian Hospital/Cornell Medicine, Lenox Hill Hospital/Northwell Health, New York, NY, USA
[n] Department of Pathology, Federal University of Goiás (UFG), Goiás, Brazil
[o] Bone Marrow Transplant Unit, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil
[p] Department of Pediatrics, Federal University of Rio de Janeiro Medical School (UFRJ), Rio de Janeiro, Brazil
[q] Medical Genetics Service, Instituto de Puericultura e Pediatria Martagão Gesteira, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil

ARTICLE INFO

Keywords:
Neurofibromatosis type 1
Cutaneous neurofibroma
Diclofenac
Laser microporation

ABSTRACT

Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder with a prevalence of 1:3000 births and a wide variety of clinical manifestations. Cutaneous neurofibromas (cNF) are among the most common visible manifestations of NF1 and present a major clinical burden for patients. NF1 patients with cNF often report decreased quality of life, emotional well-being and physical comfort. Developing effective medical therapies for cNF has been identified as a priority for the majority of adults with NF1.

Methods: The study was an open, controlled and prospective proof-of-concept clinical trial. The topical treatment consisted of two steps: cNF microporation using a laser device followed by topical application of one drop of diclofenac 25 mg/mL on the surface of the cNF (T neurofibroma = treatment) or physiological saline (C neurofibroma = control) and reapplied twice daily for 3 days. Neurofibroma assessments included visual and dermatoscopy observations noting color and presence of necrosis, presence of flaccidity, measurements in two dimensions, photographs, and histopathology after excision. The primary efficacy variable was the presence of tissue necrosis. The primary safety variable was the occurrence of treatment-related adverse events.

* Corresponding author.
E-mail address: lisa.j.brauer@gmail.com (L.B. Oliveira).
[1] Av. Brg. Trompowski, 20 - Maré, Rio de Janeiro, RJ, Brazil 21044-020.
[2] Av. Ataulfo de Paiva, 135/1104, Rio de Janeiro, RJ, Brazil 22440-901.
[3] Av. Marquês do Paraná, 303 - 2º - Centro, Niterói, RJ, Brazil 24220-000.
[4] R. Santa Luzia, 206 - Centro, Rio de Janeiro - RJ, Brazil 20020-022.
[5] 333E 38th St 4th Floor, New York, NY 10016, United States.
[6] 220East 69th Street, New York, NY 10021, United States.
[7] R. Geraldo Belo da Silva, 121-177 - Universitário, Catalão - Goias, Brazil 75705-220.
[8] Rua Galileu Galilei, 1800, 2º andar, Jd. Irajá Ribeirão Preto - São Paulo, Brazil CEP: 14020-620.

https://doi.org/10.1016/j.heliyon.2021.e06518
Received 16 April 2020; Received in revised form 16 October 2020; Accepted 11 March 2021

Results: Six patients were included in the study. The treatment resulted in transitory topical changes (healing of the microporation grid with formation of scintillating tissue layer, hyperemia and desquamation), with no statistically significant variation in the dimensions of the T and C neurofibromas in relation to pretreatment measurements. There was no necrosis in the T or C neurofibromas. In the histopathological analysis, there was no significant difference in the distribution of chronic (lymphocytic) inflammatory infiltrate in the papillary reticular dermis (subepithelial), type of infiltrate (diffuse, perivascular, or both), presence of fibrosis, and presence of atrophy among the T and C neurofibromas. No adverse events attributable to the use of diclofenac were reported during the treatment period.
Conclusions: Treatment did not result in significant alterations in terms of presence of tissue necrosis, size, or histopathological features in the T neurofibromas or in comparison to the C neurofibromas. Topical diclofenac with laser microporation was well-tolerated, with no adverse events attributable to diclofenac reported. Whether these observations are due to minimal systemic and neurofibroma exposure remain to be explored in dosage studies with larger patient groups.
Trial registration: ClinicalTrials.gov (NCT03090971) retrospectively registered March 27, 2017.

1. Introduction

Neurofibromatosis type 1 (NF1) is an autosomal dominant neurocutaneous syndrome with complete penetrance, a wide variety and range of clinical manifestations, and a worldwide prevalence of approximately 1/3000 individuals [1]. The disorder is caused by mutations of the *NF1* gene, which encodes for the protein neurofibromin. *NF1* is a tumor suppressor gene and its protein product, neurofibromin, plays a vital role in the inactivation of Ras signaling pathway. Thus, mutations of the *NF1* gene, in particular *NF1* bi-allelic inactivation, lead to a loss of neurofibromin activity which in turn increases Ras activity, permitting uncontrolled cellular proliferation and tumor formation [1, 2]. Diagnostic criteria for NF1 as established by The National Institutes of Health (NIH) Consensus Development Conference, require two or more of the following: six or more café au lait macules; two or more neurofibromas *or* one plexiform neurofibroma; axillary/groin freckling; optic glioma; two or more Lisch nodules; bony dysplasia; and a first-degree relative with NF1 [3]. As such, individuals with NF1 are often prone to develop numerous sequelae including optic glioma, intracranial tumors, kyphoscoliosis, tibial bowing, language and learning delay, hypertension, leukemia, and sarcoma [4].

Cutaneous neurofibromas (cNFs) are the most common tumor in NF1 and are present in greater than 99% of adult patients [5, 6]. cNFs are described as slow-growing lesions that involve the epidermis and dermis, are present in late childhood and generally increase in size and number with age [5, 7]. cNFs consist of soft connective tissue arising from cells in the peripheral nerve sheath and are comprised of a mixture of Schwann cells, fibroblasts and mast cells [4]. NF1-related cNFs can vary widely among affected individuals in terms of number, size, location, and distribution [8, 9]. The number of cNFs can range from just a few to several thousand, occurring at greater concentrations on the torso [4]. Neurofibromas are clinically classified as cutaneous if they occur in the skin and if, when moved, the cNF moves together with the skin over the tumor [10]. Although cNFs are histologically benign tumors [11], they can cause significant cosmetic disfigurement, pain, pruritis, and emotional and physical discomfort, and thus are directly associated with decreased quality of life of individuals with NF1 [5,11].

Current treatment options for cNF are limited to include physical removal by procedural methods, including conventional surgical resection, electrodesiccation, laser-based treatments (laser photocoagulation, CO_2 laser), and radiofrequency ablation. These approaches are limited by the number of neurofibromas that can be treated at a single surgical session, variable scarring (due to skin type and closure technique), and the time required for the procedure and wound healing [9]. Developing effective medical therapies for cNF is a priority for the majority of adults with NF1 [9,11].

Cutaneous neurofibromas are dependent on continuously activated Ras driving cellular proliferation and thus may be susceptible to treatments that target components of the Ras pathway leading to an interruption of the cell cycle or an inhibition of cellular proliferation [9].

The superficial location of cNFs renders them susceptible to topical treatment. The ideal topical cNF treatment would involve a compound that induces the involution of the cNF without scarring and/or prevents cNFs from growing in size. Diclofenac is a widely-prescribed nonsteroidal anti-inflammatory drug (NSAID) that is effective in the treatment of conditions including rheumatoid arthritis, osteoarthritis, and gouty arthritis, as an analgesic in painful conditions, and as an antipyretic in infection-associated fever [12, 13, 14]. The mechanism of action is not fully understood but diclofenac is known to be involved in inhibition of prostaglandin biosynthesis through inhibition of cyclooxygenase (COX) enzyme isoforms [15, 16]. These isoforms include cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), which catalyze the conversion of arachidonic acid into prostaglandins [14]. COX-1 is constitutively expressed in most tissue types and catalyzes the production of prostaglandins that are involved in many physiological functions, including maintenance of normal renal function, mucosal protection in the GI tract and the mediation of normal platelet function pro-aggregatory thromboxane A2 in the platelets [14, 17]. On the other hand, COX-2 expression can be induced in inflammatory cells and in human tumors by cytokines and tumor promoters [18] and is believed to have a role in the mediation of pain, inflammation, and fever [15, 17].

Topical diclofenac sodium 3% uses a gel vehicle to deliver the drug to the epidermis and is indicated in the treatment of actinic keratosis (AK), atypical neoplastic lesions that arise in sun-damaged skin that have the potential to develop into squamous cell carcinoma [19]. The mechanism of action of topical diclofenac 3% gel is unknown, however, the drug has been shown in animal models to reduce inflammation, induce apoptosis [19], inhibit angiogenesis and induce neovascular regression in inflammatory tissue [14, 20]. Due to the fact that cutaneous neurofibromas are benign lesions that rely on cellular proliferation, diclofenac could potentially inhibit neurofibroma growth in a similar manner to which it inhibits AK growth and stimulates involution. By administering the diclofenac locally to cNFs, it may result in an effective treatment with minimal cosmetic alteration and maximum safety.

Topical drug delivery aims to provide well-tolerated and effective targeted treatments and is developed based on the drug's pharmacokinetic profile and ability to penetrate the site of action. Compared to oral administration, topical drug delivery offers the advantages of bypassing first-pass metabolism and a decreased incidence of systemic adverse effects, while allowing for direct application to target locations and thus providing acceptability and ease of use. In order to guarantee a therapeutic effect of a topical formulation, the drug must be able to both penetrate the skin and permeate target areas at sufficient concentrations to exert a therapeutic effect [21].

In a previous study, we evaluated the safety and efficacy of intralesional administration of diclofenac (Voltaren® injection 75 mg/3mL ampoule) on cNFs. In this study, some treated cNFs experienced inflammation, tissue necrosis, and full or partial detachment. These events were not observed in the control cNFs [22]. These findings justify

additional investigation into the efficacy of using diclofenac to treat cNFs in the search for the most convenient drug delivery system.

Although cNFs are easily accessible lesions, the formulation of topical diclofenac gel would likely be unable to adequately penetrate these benign tumors because of the barrier function of the skin's corneal layer, a known limiting factor for passive drug penetration and transdermal drug delivery [23]. Laser microporation followed by topical application of liquid diclofenac may offer an alternative delivery method, while providing the benefit of administering a significantly smaller dose of diclofenac as compared to oral or intralesional treatment [22]. Furthermore, topical application of the treatment may be applied at home by the patient painlessly and at less final cost to the patient, with greater safety. Clinical studies have shown that topical administration resulted in higher diclofenac levels in the dermis as compared to oral administration, providing further evidence for the efficacy of local administration [24, 25]. However, the excellent barrier function of the stratum corneum limits the rate and extent of topical drug delivery [26, 27], and focus has moved to the development of new technologies to reversibly impair barrier function and so enhance transport [28].

Lasers have been used for medical applications for more than 50 years. Over the past 20 years, lasers have come to represent an invaluable tool in dermatological treatment due to their ability to penetrate and act through tissue without causing bleeding [29]. Microporation is a method currently used as part of the treatment of various skin conditions such as actinic keratosis and was used to enhance delivery of topical administered substances such as diclofenac [23]. Researchers have illustrated that laser microporation, using P.L.E.A.S.E.® technology, significantly increased diclofenac transport. This option is especially helpful for lesions in small and restricted areas [29]. P.L.E.A.S.E. (Precise Lasers Epidermal System) from Pantec Biosolutions AG (Liechtenstein) is a medical device based on an Er:YAG laser to generate micropores in the skin. The device emits light with a wavelength of 2.94 μm and therefore breaks the stratum corneum by creating aqueous micropores, with variable user settings allowing for variation in micropore number, density, and depth [23]. This study evaluated the use of P.L.E.A.S.E. platform cutaneous neurofibroma microporation followed by topical diclofenac application.

2. Methods

2.1. Objectives

The objectives of this study were to evaluate the efficacy and safety of using topical diclofenac 25 mg/ml in the treatment of cutaneous neurofibromas in individuals with NF1.

2.2. Study design and population

The study was an open, controlled, prospective, proof-of-concept clinical trial conducted at UNIFESO Medical School in the State of Rio de Janeiro, Brazil. The study protocol received local ethical committee approval from the institution (approval no. 925.891) and is registered at ClinicalTrials.gov (ID: NCT03090971). All participants provided written informed consent prior to data collection.

The inclusion criteria included patients with a clinical diagnosis of NF1 according to the NIH Consensus Development Conference NF1 diagnostic criteria (NIH, 1998) and had four or more cNFs measuring 0.5–1.2 cm in greatest diameter present on either the thorax, abdomen, and upper or lower limbs (measured in a single plane). In keeping with the previous study [22], seven patients initially satisfied these inclusion criteria and six were enrolled in the study (one patient was unable to participate due to an unrelated family matter).

2.3. Treatment

The study period lasted a total of 37 days and consisted of three treatment visits and three follow-up visits to the study center: Visit 1 (Pretreatment/Day 1), Visit 2 (Day 4), Visit 3 (Day 7), Visit 4 (Day 10), Visit 5 (Day 17) and Visit 6 (Day 37). The schedule of study assessments is shown in Table 1.

Two cNFs on each patient were designated as treatment (T) or control (C) neurofibromas: T1/C1 measuring between 0.5-0.8cm and T2/C2 measuring between 0.81-1.2cm. Each cNF was marked with a subject identification number (001, 002, 003, 004, 005, 006) and neurofibroma number (1, 2) using a marker with black ink. Each cNF was photographed three times at each visit, using a digital camera with a minimum resolution of 800 × 600 and macro capacity with a zoom lens of 35–105mm. Photographs were taken at a 90° angle from the specified cNF. The angle of exposure was maintained in all photographs.

During Visits 1, 2 and 3, cNF underwent laser microporation and were treated with diclofenac or saline solution on Day 1, Day 4 and Day 7, respectively. cNFs were laser-porated using the P.L.E.A.S.E.® device with the following user-defined settings: density of 10%, 5 pulses per pore, repetition rate of 300 Hz, pulse duration of 125 μs, pore array size of 10mm and fluence of 36.2 J/cm^2. After microporation, researchers topically applied one drop of diclofenac 25 mg/ml (Voltaren®) to the surface of the T neurofibromas (T neurofibroma = treatment) or physiological 0.9% saline solution to the surface of C neurofibromas (C neurofibroma = control). Patients were then directed to re-apply the respective study medication twice daily for three days at home following instructions given during Visit 1 on how to locate each neurofibroma and apply the study medication. Subjects were instructed to return to the study center at three-day intervals for another round of laser microporation and topical application, followed again by at-home topical application for three more days.

After the treatment period, Visits 4, 5 and 6 solely consisted of the follow-up assessments on Day 10, Day 17 and Day 37, respectively. Therefore, the three follow-up assessments took place 3, 7, and 30 days after the final round of laser microporation. At the researcher's discretion, additional follow-up visits were scheduled to evaluate the neurofibroma healing process.

The T and C neurofibromas of all participating subjects were surgically removed at the end of the study (at the end of Visit 6), fixed in 10% buffered-formaldehyde, and sent for histopathological analysis. Samples were handled and processed according to routine histological procedures and a 5-μm hematoxylin & eosin section was used for morphological analysis. The histopathological evaluations were performed by two independent pathologists.

2.4. Measurements & data collection

Primary outcome measures indicating clinical efficacy were the presence of an inflammatory process and tissue necrosis in T neurofibromas. Secondary outcome variables included the reduction in T neurofibroma size or detachment of T neurofibromas. The primary endpoint for the study was the presence of necrosis on T neurofibromas. Secondary endpoints included change in color, shape, and structure of the T neurofibromas and occurrence of adverse events (including any significant changes to laboratory exams).

At each visit, subjects underwent serial physical examinations, recording of adverse events, and assessment of both T and C neurofibromas. The assessment of T and C neurofibromas included visual and dermatoscopy observations (using the Dermlite DL3 [3Gen, San Jan Capistrano, CA, USA]) noting color and presence of necrosis, presence of flaccidity, and size. The neurofibromas were measured in two axes using a measuring tape: (x [horizontal] and y [vertical]).

Patient safety was evaluated by adverse event monitoring and dermatological assessments of local and global skin reactions considered to be associated with topical diclofenac application during the 7-day treatment period and 30-day follow-up period. Adverse event grading followed that of the previous study: Mild (conscious of the event, but easily tolerated); Moderate (sufficient discomfort to interfere with daily activities); Severe (inability to perform daily activities). Primary outcome

Table 1. Schedule of study assessments.

Visit	V. 1 *Baseline, treatment day 1*	V. 2 *After 3 days of treatment*	V. 3 *Three days after V.2*	V. 4 *Three days after V.3*	V. 5 *Seven days after V.4*	V. 6 *30 days after V.3*
Study day	1	4	7	10	17	37
Informed consent	x					
Medical history	x					
Physical exam and vital signs	x	x	x	x	x	x
Laboratory tests	x		x			x
Neurofibroma assessments	x	x	x	x	x	x
Neurofibroma photographs	x	x	x	x	x	x
Adverse event monitoring		x	x	x	x	x
Concomitant medication monitoring	x	x	x	x	x	x
Laser microporation	x	x	x			
Topical diclofenac application	x——————————x					
Neurofibroma resection/histopathology						x

measures indicating safety of topical use was the occurrence of an adverse event. Secondary outcome measures included data from clinical evaluations (physical examination and vital signs), severity and duration of adverse events, and any changes in laboratory parameters.

During Visit 1, a full medical history was obtained, a complete physical examination, including vital signs, were recorded, concomitant medications used within 30 days of inclusion were noted, and laboratory evaluations were performed in order to evaluate the safety of topical diclofenac use in each participating subject. In addition to Visit 1 (pre-treatment), laboratory evaluations were also performed during Visits 2, 3, and 6 at the same laboratory (Laboratório Richet, Rio de Janeiro, Brazil). The laboratory evaluations included a urinalysis, complete blood count, liver function tests (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase, total and direct bilirubins), basic metabolic profile (urea, creatinine, sodium, and potassium) and magnesium. Throughout the duration of the 37-day study period, concomitant medication use was monitored during each subsequent visit. Adherence to study protocol was evaluated at Visit 2, 3 and 4.

2.5. Statistical analysis

Data was collected on a clinical research form and analyzed using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego California USA). Frequency tables were generated and central tendencies (mean, median, mode) were calculated. Overall clinical efficacy and safety were statistically analyzed by comparing the results of Visits 2–6 with in relation to pretreatment values at Visit 1. Categorical variables were analyzed using chi-squared or Fisher's Exact test was used, while continuous variables were analyzed with repeated measures ANOVA or Student's T-test with a significance level of 0.05 and 95%CI.

3. Results

3.1. Study population and pretreatment characteristics

A total of six subjects were screened, enrolled, and completed the study from February–March 2017: one man (17%) and five women (83%); mean age, 46.17 years (±16.44). The six participants were included in analysis of each objective. At pretreatment neurofibroma selection and assessment, the T and C neurofibromas were described as skin color, with no significant alterations prior to initiation of treatment. No significant alteration in physical exams was noted at pretreatment.

3.2. Compliance & concomitant medications

Apart from one exception, compliance to treatment regimen was good and patients confirmed their facility to follow instructions for application given at pretreatment. Subject 005 skipped one diclofenac and serum application on the T and C neurofibromas between Visit 2 and Visit 3.

One subject reported use of concomitant medications at Visit 1 which continued throughout the study period (omeprazole 20mg, ranitidine 150mg, scopolamine butylbromide 10mg). Concomitant medications reported during the treatment period included paracetamol 750mg ($n = 1$) and 500mg ($n = 1$), metamizole 500mg ($n = 1$), metamizole 300mg + caffeine 35mg + orphenadrine 500mg ($n = 2$), amoxicillin 500mg ($n = 1$), tenoxicam 20mg ($n = 1$), loratadine 10mg ($n = 1$), and ferrous sulphate 150mg ($n = 1$).

3.3. Physical exam

There were no significant alterations in the physical exam and vital signs performed at each study visit in relation to pretreatment observations (ANOVA p values > 0.05 for weight, blood pressure, pulse, and temperature).

3.4. Laboratory exams

At pretreatment, one subject had moderate anemia on CBC (persisting subsequent visits) and one subject had slightly low ALT, persisting at Visits 3 and 6. At Visit 3, one subject had slightly low alkaline phosphatase (persisting at Visit 6). At Visit 6, one subject presented elevated urea and C reactive protein (requested due to adverse event), two subjects had slightly low alkaline phosphatase, and one subject had slightly low mean corpuscular hemoglobin concentration (MCHC).

3.5. Neurofibroma assessment

3.5.1. Necrosis

None of the T or C neurofibromas presented necrosis during the treatment period or upon histopathology examination.

3.5.2. Color and observations

At Visit 1, the color of all T and C neurofibromas was described as “skin color”, without other pretreatment alterations.

At Visit 2, on visual observation and dermoscopy, all T and C neurofibromas displayed healing in the shape of the laser microporation grid on the surface of the neurofibromas.

At Visit 3, on visual observation and dermoscopy, all T and C neurofibromas displayed healing in the shape of the laser microporation grid on the surface of the neurofibromas. On dermoscopy, hyperemia and shining dots around the micropores were noted (epithelialization), with some neurofibromas presenting desquamation.

At Visit 4, hyperemia and desquamation of T and C neurofibromas were apparent on visual observation and on dermoscopy, where white

shining dots around the micropores and around the surface of the neurofibromas were noted (epithelialization).

At Visit 5, hyperemia and shiny areas in the presence of microporation healing (epithelialization) were noted on visual observation and dermoscopy.

At Visit 6, on visual observation and dermoscopy, neurofibromas were pallid or unaltered in relation to Visit 1, with hyperemia persisting in some neurofibromas.

There were no statistically significant differences in the number of T and C neurofibromas presenting hyperemia ($\chi^2 = 8.437$; DF = 12; p = 0.7501 for T and C neurofibromas; $\chi^2 = 5.5$; DF = 9; p = 0.789 for T neurofibromas, and $\chi^2 = 3.378$; DF = 9; p = 0.947 for C neurofibromas) or pallidity ($\chi^2 = 0.1048$, DF = 12; p = 1.0 for T and C neurofibromas; $\chi^2 = 2.019$; DF = 5; p = 0.847 for T neurofibromas, and $\chi^2 = 0.0$; DFL = 5; p = 1.0 for C neurofibromas).

3.5.3. Flaccidity

The presence of flaccidity in the T and C neurofibromas at each study visit is displayed in Table 2. The number of neurofibromas presenting flaccidity from Visit 2 to Visit 6 did not reach statistical significance for the T ($\chi^2 = 2.956$; DF = 5; p = 0.7068) or C ($\chi^2 = 0.7132$; DF = 5; p = 0.9822) neurofibromas.

3.5.4. Neurofibroma photographs

Figures 1, 2, 3, and 4 display sequential photographs of T and C neurofibromas throughout the study.

3.5.5. Neurofibroma size

Changes in T and C neurofibroma sizes in two axes are presented in Table 3.

3.5.6. Histopathology features

Histopathological analysis of the T and C neurofibromas analyzed between 6 and 32 sequential slices from each tumor, confirmed all to be cutaneous neurofibromas, not encapsulated, confined in the dermis. Some had diffuse limits (5/6 for T1, C1, and C2 neurofibromas) and others had a well-defined delimitation (1–6 for C2 neurofibroma), with one T1 neurofibroma described as diffuse, but well limited in lower depth.

Upon assessment of the T and C neurofibroma epidermis, atrophy was observed (T1: 5; T2/C1/C2: 4), as well as atrophy with hyperkeratosis (T1: 1; T2: 2; C2:1), while two C1 neurofibromas and one C2 neurofibroma showed no epidermal alterations. There was no statistically significant difference in the number of neurofibromas presenting each type of observation during evaluation of the epidermis ($\chi^2 = 5.843$; DF = 6; p = 0.4410).

Five of the T1 neurofibromas and all 6 T2 neurofibromas presented superficial, mild to moderate intratumoral lymphocytic chronic inflammatory infiltrate, also present in 4/6 C1 neurofibromas and 3/6 C2 neurofibromas. There were no statistically significant differences in the number of T and C neurofibromas presenting intratumoral inflammatory infiltrate ($\chi^2 = 4.444$; DF = 3; p = 0.2173). With the exception of one C1 neurofibroma, all study neurofibromas presented mild to moderate lymphocytic chronic inflammatory infiltrate on the papillary and reticular dermis (subepithelial), with diffuse, perivascular, or perivascular/diffuse distribution (however there was no statistically significant difference between T and C neurofibromas in distribution [$\chi^2 = 7.176$; DF = 9; p = 0.6188]).

Fibrosis was present in the papillary and reticular dermis in all T neurofibromas (with intratumoral fibrosis noted in two T1 neurofibromas and superficial fibrosis noted in three T1 neurofibromas and in three T2 neurofibromas) and in 4/6 C1 and C2 neurofibromas (one C1 and two C2 neurofibromas presenting superficial fibrosis), with no statistically significant difference between the T and C neurofibromas in terms of fibrosis presence ($\chi^2 = 4.8$; DF = 6; p = 0.1870).

3.6. Adverse events

During Visit 1, one subject reported pain during T1 neurofibroma microporation, lasting a few seconds; this same subject also reported burning sensation after microporation and saline solution application on C1 neurofibroma, lasting 4 min. During Visit 2, one subject reported burning sensation after microporation and saline solution application on both C neurofibromas, lasting 8 min. Also, at Visit 2, one patient reported pain during microporation of the C1 neurofibroma lasting a few seconds. At Visit 4, one patient reported bleeding of C1 neurofibroma after friction with a bath towel.

The adverse events not directly related to microporation and treatment application are summarized in Table 4. No serious adverse event was reported during the study, and no adverse event occurred causing treatment withdrawal.

4. Discussion

To our knowledge, this is the first study using topical diclofenac for the treatment of cutaneous neurofibromas. Topical administration of diclofenac and other drugs is an alternative to oral administration offering potential advantages of greater ease of application and a more favorable safety profile [30], and, in the case of cNF treatment, an alternative to intralesional treatment [22]. However, in order for the efficacy of topical administration of a drug to be comparable to other routes of administration, it is necessary for the drug to have the ability to penetrate the dermis and present a permeation at the target site at concentrations sufficient for a therapeutic effect to be established. The factors to be considered for this to occur include the physicochemical properties of the drug itself, the formulation used, the site of application and the mode of application [21]. It is possible that the permeation of diclofenac at the concentration used in the present study and in the liquid formulation did not reach a level sufficient to trigger the previously observed changes [22], given that the absence of necrosis in the T neurofibromas was a an unintended finding, based on the results of the study by Geller et al. (2015), where whole neurofibromas presented necrosis and detachment during the intralesional treatment period with diclofenac at 25mg/ml [22]. This result led to the use of this outcome as the primary endpoint in the present study.

Although there was some size reduction in the X- or Y-axis relative to pre-treatment of the T neurofibromas (50% of neurofibromas showing reduction on X-axis and 58.3% presenting reduction on Y-axis) and C

Table 2. Distribution of T and C neurofibromas presenting flaccidity at each study visit.

Neurofibromas presenting flaccidity	Visits					
	V. 1	V. 2	V. 3	V. 4	V. 5	V. 6
T1	0	1	4	6	6	5
T2	0	5	6	5	6	4
C1	0	3	2	4	5	2
C2	0	5	4	5	6	5

Data are *n*.

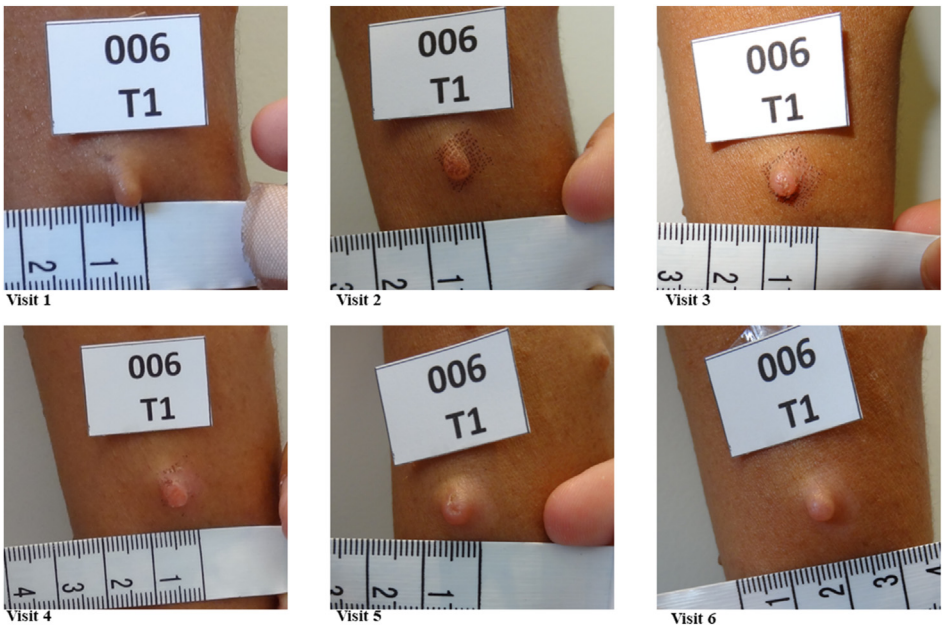

Figure 1. Sequence of photographs showing a T1 neurofibroma at each study assessment visit. Note Microporation grid at Visit 2 and subsequent desquamation. Neurofibroma and microporation grid appear pallid at Visit 6 in relation to pretreatment. (006 T1).

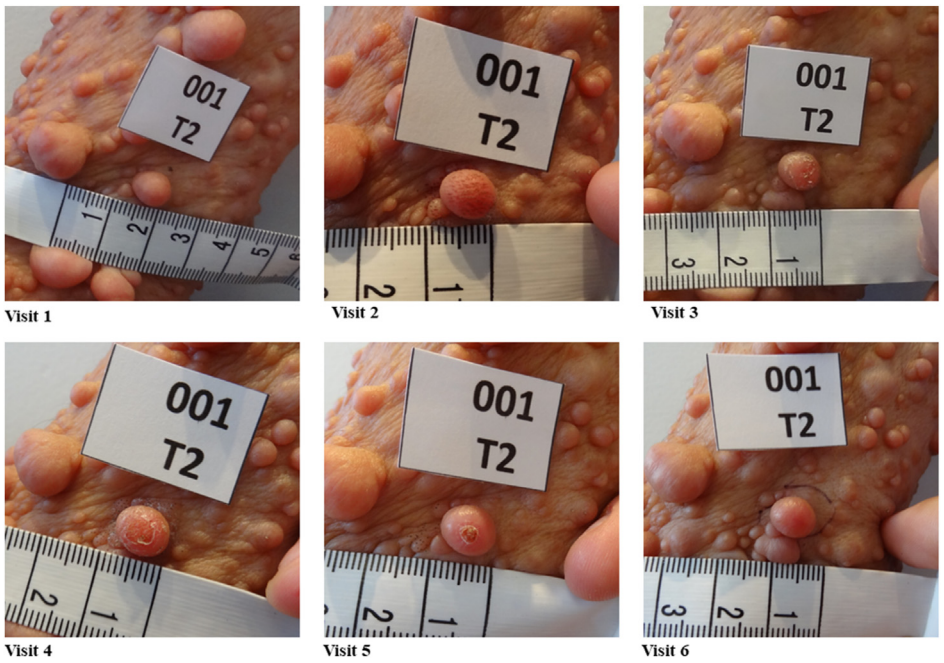

Figure 2. Sequence of photographs showing a T2 neurofibroma at each study assessment visit. Note Microporation grid at Visit 2 and subsequent desquamation. Neurofibroma appears unaltered at Visit 6 in relation to pretreatment (001 T2).

neurofibromas (66.7% of neurofibromas with reductions on X-axis and 50% with reduction on the Y-axis), there were few treated neurofibromas with statistically significant changes in the size. Measurement of neurofibromas is a challenge in clinical assessments of treatments for cNF in NF1 as it may be subject to considerable inter-observer variability [9, 31, 32]. The size of a cNF may be indicative of a clinically significant result for therapeutic success evaluations, however even the use of the caliper, which was considered a reliable method in a recent study of natural history [33], has limitations in the measurement of small or flat tumors and does not evaluate the non-visible portions of the neurofibroma [32]. The reductions observed in the size of the neurofibromas T and C could be associated with the healing process of the microporation through the formation of fibrosis. As noted above, the absence of the expected necrosis in this study was probably due to the lower antiangiogenic action of diclofenac (likely due to non-occurrence of the anti-angiogenesis observed when using injectable form intralesionally).

Superficial changes in the neurofibromas were observed during the treatment period (color changes, microporation grid healing, and desquamation), but were generally not present in the final evaluation. The formation of a shiny tissue layer observed at Visits 3, 4, and 5 have previously been described in the literature and is documented photographically in the manufacturer's internal documents as part of the

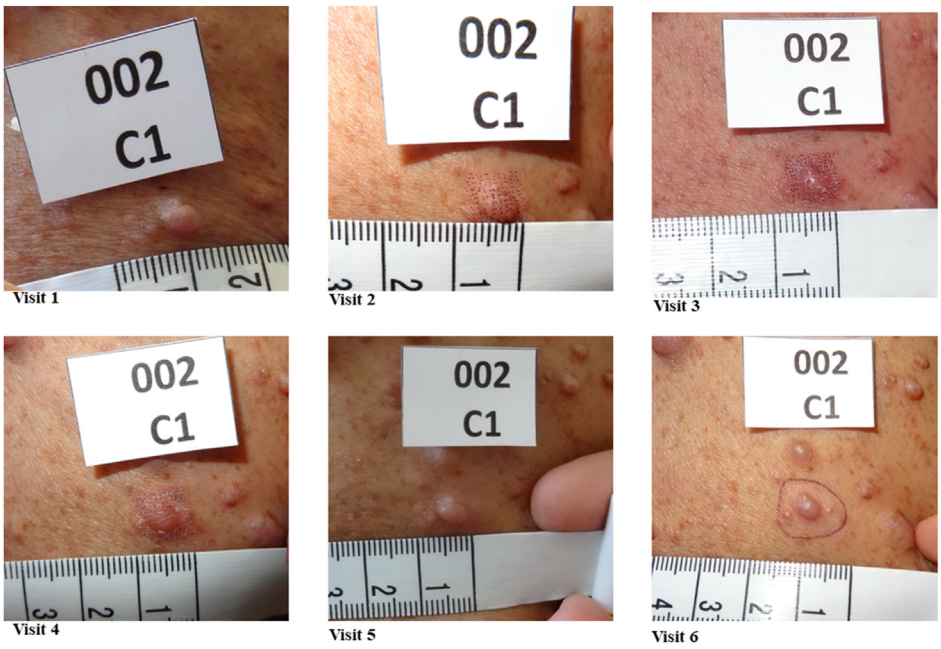

Figure 3. Sequence of photographs showing a C1 neurofibroma at each study assessment visit. Note Microporation grid at Visit 2 and subsequent desquamation. Neurofibroma appears unaltered at Visit 6 in relation to pretreatment (002 C1).

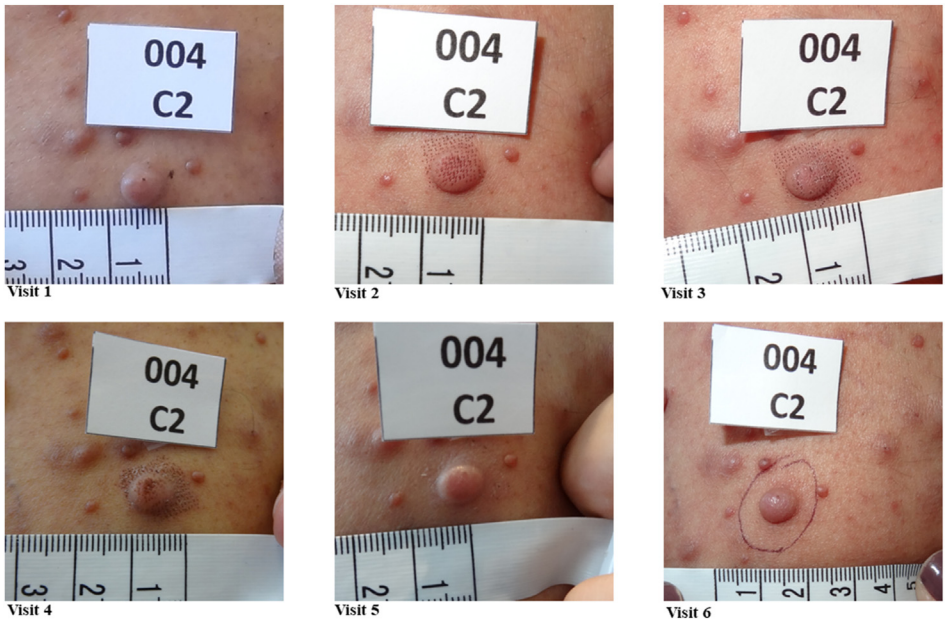

Figure 4. Sequence of photographs showing a C2 neurofibroma at each study assessment visit. Note Microporation grid at Visit 2 and subsequent desquamation. Neurofibroma appears unaltered at Visit 6 in relation to pretreatment (004 C2).

skin post-microporation healing process in patients not affected by NF1 [34]. No previous references have been found in the literature on the use of laser microporation in neurofibromas of patients with NF1.

It is likely that hyperemia and desquamation also occurred as part of the microporation healing process, taking into account repeated microporation on the same surface. The micropores generated by the device P.L.E.A.S.E.® occur as a function of the emission of light at the absorption wavelength of 2.94 μm, resulting in the excitation and explosive evaporation of the water molecules present in the epidermis [35]. In 2012, Brogden *et al.* reported a delay in the closure of micropores treated with diclofenac gel (Solaraze® - containing 3% diclofenac sodium and 2.5% hyaluronic acid) in relation to the control, after microporation using microneedles measuring 800 μm in length and 200 μm in width at the base, on human skin [36]. The absence of large differences observed between neurofibromas T and C at Visits 2, 3 and 4 could be indicative of the absence of this effect with laser microporation and the use of diclofenac in liquid form, although it is also important to point the use of occlusive adhesive in study of Brogden *et al.*, which was not used in the present study. The changes observed in neurofibromas did not generally result in altered neurofibroma size and there was no evidence of necrosis during treatment, it being possible that the transient nature of the microporation-induced alteration followed by diclofenac topical application was insufficient to induce the necrosis observed in the previous study.

Table 3. Change in T and C neurofibroma measurements at Visit 6 in relation to Pretreatment measurements.

T1			
Subject	x	y	Significant change?
001	No change	Reduction	No (p = 0.5)
002	No change	Reduction	No (p = 0.822)
003	No change	No change	No (p = 0.5)
004	No change	Reduction	No (p = 0.5)
005	Reduction	Reduction	No (p = 0.366)
006	No change	Reduction	No (p = 0.5)
T2			
001	Reduction	No change	No (p = 0.187)
002	Reduction	No change	No (p = 0.127)
003	Reduction	No change	No (p = 0.5)
004	Reduction	Reduction	Yes (p = 0.0047)
005	Reduction	No change	No (p = 0.5)
006	Increase	Increase	No (p = 0.051)
C1			
001	Reduction	Reduction	Yes (p = 0.038)
002	Increase	Reduction	No (p = 0.62)
003	Reduction	No change	No (p = 0.5)
004	Reduction	Reduction	No (p = 0.5)
005	Reduction	Reduction	No (p = 0.142)
006	No change	No change	Yes (p = 0.038)
C2			
001	Reduction	Reduction	Yes (p = 0.038)
002	Reduction	No change	No (p = 0.822)
003	No change	No change	No (p = 1.0)
004	Reduction	Increase	No (p = 0.977)
005	Reduction	Increase	No (p = 0.648)
006	No change	Reduction	No (p = 0.5)

Note: x = horizontal measurement (measured in cm at each study visit; y = vertical measurement (measured in cm at each study visit)).

Table 4. Adverse Events occurring during study treatment period.

Subject	Diagnosis	Severity	Continuing at end of study?	Duration	Relation to treatment
1	Headache	Moderate	No	5h	Unknown
1	Headache (menstruation)	Moderate	No	4h	Unknown
1	Headache	Moderate	No	1d	Unknown
2	Headache	Moderate	No	5h	Unknown
2	Headache	Moderate	No	0.5h	Unknown
2	Bilateral heel edema. Well-defined, hyperemic, pruritic (more pronounced at night) (lesions 1cm in circumference), painful on deambulation. Diagnostic hypothesis: parasitic infection	Moderate	Yes	1d	Unknown
4	Somnolence	Mild	No	3h	Unknown
4	Headache	Severe	No	2h	Unknown
5	Lumbago	Moderate	No	8h	Unknown
6	Sinusitis	Moderate	No	1d	Unknown
6	Coryza	Moderate	No	1d	Unknown
6	Sore throat	Moderate	No	1d	Unknown
6	Ear ache	Moderate	No	1d	Unknown
6	Increased body temperature	Mild	No	1d	Unknown

Note: h = hours; d = days.

The use of the dermatoscope was of great value in the neurofibroma assessments, allowing observations with greater details of the surface of the neurofibromas. At some times, what appeared to be necrosis points on the surface of the neurofibromas were observed visually, but when viewed through the dermatoscope, it was possible to determine that the observed point was actually the formation of a crust during healing. Dermoscopy was suggested as a tool for diagnostic confirmation of cutaneous neurofibromas in the outpatient setting by Numan and Elmas (2015), who described the characteristics of cutaneous neurofibromas associated with café-au-lait spots [37].

Flaccidity was described in the previous study in T and C neurofibromas [22], which is why it was included as a variable in this study. It was considered a sign of some alteration occurring in the neurofibroma, however, the observation of flaccidity in the C neurofibromas suggests that this effect was not caused by the diclofenac applications. Simultaneously, we cannot correlate this effect with the laser microporation, as flaccidity was not universally observed in the microporated neurofibromas.

The histolopathological evaluation revealed intratumoral and subepithelial chronic inflammatory infiltrate in the neurofibromas, as well as atrophy and fibrosis, but there was no significant difference in T neurofibromas in relation to the C neurofibromas. Therefore, it remains uncertain which observations may be associated with the microporation itself and which could be associated with the additional use of diclofenac. The atrophy present in the epidermis of all neurofibromas (with the exception of one T1 neurofibroma and one C2 neurofibroma) is described in the literature as a possible finding in the routine histopathological analysis of cutaneous neurofibroma [38].

The fibrosis present in the neurofibromas (all T neurofibromas and 4/6 C1 and C2 neurofibromas) could be due to the microporation, since fibrosis occurs during the healing process [39]. The presence of fibrosis in all T neurofibromas may be indicative of an influence of diclofenac during the microporation healing process, and the variations in the presence of fibrosis among the neurofibromas C could be related to the healing process of the microporation, but may also be due to the histopathological variations between cutaneous neurofibromas even from a single patient. Some present a loose stroma with fine collagen fibers, while others have a denser stroma. Moreover, variations on histopathological features may also occur during the neurofibroma evolution; in longstanding tumors, there may be heavy collagen deposition [40].

In the study by Geller et al. (2015), histopathological analysis performed after resection of treated neurofibromas revealed intratumoral infiltration of neutrophils and lymphocytes, with evident necrosis accompanied by thrombosis, and these observations were not recorded in the control neurofibromas [22]. In contrast, in the present study, intratumoral lymphocytic chronic inflammatory infiltrate was present in 5/6 of the T1 neurofibromas and all 6 T2 neurofibromas, but was also present in 4/6 C1 neurofibromas and 3/6 C2 neurofibromas, while in this study no thrombosis or necrosis was observed in any of the neurofibromas. The difference in the findings between the two studies can be accounted for by the application method used in the present study. Future studies should also describe the composition of the inflammatory infiltrate in detail, considering studies demonstrating CD4/CD8+ T-cells and PD-1 expression on tumor cells [41].

It should be noted that no adverse effects attributable to the use of diclofenac during the treatment period were observed in this study. The occurrence of necrosis, despite being an expected and desired result in this study, has also been described as an adverse effect of diclofenac sodium following subcutaneous and intramuscular application in the treatment of other conditions not related to NF1, such as fever [42, 43]. During microporation there were isolated reports of pain and subsequent burning sensation. However, no pain sensation is expected upon microporation performed at the configurations used, since the pores do not reach the dermis (>160 μm) [44], therefore the reports registered in the present study are likely related to anxiety on tension and expectation on the part of the subject at the time of the microporation. The perception of pain varies from person to person and is subject to several mental processes beyond nociceptive stimulation, including emotional factors, expectations, and individual beliefs [45].

Although not significant, the results of this study were valuable in indicating the need to find an alternative delivery system for the treatment of cutaneous neurofibromas with diclofenac. Some recommendations based on our experience and the limitations encountered in this study may be of use in the future.

The shape of the laser applicator allows the creation of a surface microporation grid, which, depending on neurofibroma size, does not reach the base of the tumor. The applicator also does not allow the visualization of the neurofibroma during the application of the laser, and any movement by the patient or the technician applying the laser results in a non-centralized grid, which happened during the treatment during the first microporation. In applications in endocrinology, the device is the same, however the goal is to microporate the skin to facilitate drug penetration, and such focused precision of the microporation site is not required as it was for the treatment of individual cutaneous neurofibromas. The manufacturer has indicated the possibility of developing an applicator with the necessary adjustments to improve visualization during the application and also to reach the base of the neurofibroma.

To maintain uniformity between patients and neurofibromas, measurement of neurofibromas took into account a measure with caliper and tape measure in a horizontal position in relation to the ground and another in a vertical position in relation to the ground. However, the inclusion of neurofibroma was allowed provided it was within the largest size range (0.5–0.8cm for T1 and C1 neurofibromas and 0.81–1.2cm for T2 and C2), which was not always reached in the horizontal or vertical position in relation to the ground (eg., larger dimension allowing the inclusion of the measured neurofibroma in an inclined position).

Regarding the size of the neurofibromas evaluated in this study, following the previous study design, we evaluated neurofibromas in the size range of 0.5–1.2cm. However, it could be more useful in future studies to evaluate neurofibromas within a more limited size range, for example 0.5–0.8cm, in order to remove possible variability due to size variation and to allow for better evaluation of drug permeation.

As this study was an open study, there was no blinding. We understand the risk of bias in an unblinded study, despite all the care taken by the team during the study.

For future, larger studies, functional patient related outcome measures and quality of life assessments should also be included when evaluating a different technique (such as the laser microporation used in the present study) and drug (Diclofenac) for the treatment of non-malignant conditions.

5. Conclusions

Treatment of cutaneous neurofibromas of NF1 patients with laser microporation followed by topical application of liquid diclofenac 25 mg mL did not result in significant alterations in terms of presence of necrosis, size, or histopathological features in the treated neurofibromas in comparison to the control neurofibromas. Topical diclofenac with laser microporation was well-tolerated, with no adverse events attributable to diclofenac reported. Whether these observations are due to minimal systemic and neurofibroma exposure remain to be explored in dosage studies with larger patient groups.

Declarations

Author contribution statement

Lisa Brauer Oliveira and Mauro Geller: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Karin Soares Cunha: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Alessandra Santos: Performed the experiments.

Allan Bernacchi: Conceived and designed the experiments; Performed the experiments.

Allan E. Rubenstein, Sanyu Takirambudd, Spyros Mezitis and Luiz Guilherme Darrigo Jr: Conceived and designed the experiments; Wrote the paper.

Carolina de Almeida Ito Brum: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Marcia Gonçalves Ribeiro: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

The clinical trial described in this paper was registered at Clinical-Trials.gov under the registration number NCT03090971.

Acknowledgements

Not applicable.

References

[1] D.H. Gutmann, R.E. Ferner, R.H. Listernick, B.R. Korf, P.L. Wolters, K.J. Johnson, Neurofibromatosis type 1, Nat. Rev. Dis. Primers 3 (2017 Feb 23) 17004.
[2] B.R. Korf, Neurofibromatosis, Handb. Clin. Neurol. 111 (2013) 333–340.
[3] Neurofibromatosis. Conference statement. National Institutes of Health Consensus development conference, Arch. Neurol. 45 (5) (1988 May) 575–578.
[4] S.M. Levine, E. Levine, P.J. Taub, H. Weinberg, Electrosurgical excision technique for the treatment of multiple cutaneous lesions in neurofibromatosis type I, J. Plast. Reconstr. Aesthetic Surg. 61 (8) (2008 Aug) 958–962.
[5] K.I. Ly, J.O. Blakeley, The diagnosis and management of neurofibromatosis type 1, Med. Clin. North. Am. 103 (6) (2019 Nov) 1035–1054.
[6] S.M. Huson, P.S. Harper, D.A. Compston, Von Recklinghausen neurofibromatosis. A clinical and population study in south-east Wales, Brain 111 (Pt 6) (1988 Dec) 1355–1381.
[7] V.C. Williams, J. Lucas, M.A. Babcock, D.H. Gutmann, B. Korf, B.L. Maria, Neurofibromatosis type 1 revisited, Pediatrics 123 (1) (2009 Jan) 124–133.
[8] R.J. Allaway, S.J.C. Gosline, S. La Rosa, P. Knight, A. Bakker, J. Guinney, L.Q. Le, Cutaneous neurofibromas in the genomics era: current understanding and open questions, Br. J. Canc. 118 (12) (2018 Jun) 1539–1548.
[9] S.K. Verma, V.M. Riccardi, S.R. Plotkin, H. Weinberg, R.R. Anderson, J.O. Blakeley, et al., Considerations for development of therapies for cutaneous neurofibroma, Neurology 91 (2 Suppl 1) (2018 Jul 10) S21–S30.
[10] V.M. Riccardi, The genetic predisposition to and histogenesis of neurofibromas and neurofibrosarcoma in neurofibromatosis type 1, Neurosurg. Focus 22 (6) (2007 Jun 15) E3.
[11] N. Ortonne, P. Wolkenstein, J.O. Blakeley, B. Korf, S.R. Plotkin, V.M. Riccardi, et al., Cutaneous neurofibromas: current clinical and pathologic issues, Neurology 91 (2 Suppl 1) (2018 Jul 10) S5–S13.
[12] R.O. Day, A.J. Mclachlan, G.G. Grahm, K.N. Williams, Pharmacokinetics of non-steroidal anti-inflammatory drugs in synovial fluid, Clin. Pharmacokinet. 36 (3) (1999 Mar) 191–210.
[13] N.M. Davies, K.E. Anderson, Clinical pharmacokinetics of diclofenac. Therapeutic insights and pitfalls, Clin. Pharmacokinet. 33 (3) (1997 Sep) 184–213.
[14] T.J. Gan, Diclofenac: an update on its mechanism of action and safety profile, Curr. Med. Res. Opin. 26 (7) (2010 Jul) 1715–1731.
[15] J.R. Vane, R.M. Botting, Mechanism of action of nonsteroidal anti-inflammatory drugs, Am. J. Med. 104 (3A) (1998 Mar 30) 2S–8S, discussion 21S-22S.
[16] J.R. Vane, The mechanism of action of anti-inflammatory drugs, Int. J. Clin. Pract. Suppl. (135) (2003 Apr) 2.
[17] P.G. Conaghan, A turbulent decade for NSAIDs: update on current concepts of classification, epidemiology, comparative efficacy, and toxicity, Rheumatol. Int. 32 (6) (2012 Jun) 1491–1502.
[18] J.L. Masferrer, K.M. Leahy, A.T. Koki, B.S. Zweifel, S.L. Settle, B.M. Woerner, et al., Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors, Canc. Res. 60 (5) (2000 Mar 1) 1306–1311.
[19] G.M. Martin, E. Stockfleth, Diclofenac sodium 3% gel for the management of actinic keratosis: 10 + years of cumulative evidence of efficacy and safety, J. Drugs Dermatol. JDD 11 (5) (2012 May) 600–608.
[20] J.B. Méric, S. Rottey, K. Olaussen, J.C. Soria, D. Khayat, O. Rixe, et al., Cycooxygenase-2 as a target for anticancer drug development, Crit. Rev. Oncol. Hematol. 59 (1) (2006 Jul) 51–64. Epub 2006 Mar 13.
[21] M. Hagen, M. Baker, Skin penetration and tissue permeation after topical administration of diclofenac, Curr. Med. Res. Opin. 33 (9) (2017 Sep) 1623–1634.
[22] M. Geller, A. Bonalumi Filho, L. Oliveira, A.E. Rubenstein, L.G. Darrigo Jr., D. Azulay, et al., A proof-of-concept assessment of the safety and efficacy of intralesional diclofenac in the treatment of cutaneous neurofibromas, Int. J. Clin. Med. 6 (12) (2015) 975–983.
[23] Z. Oesterreicher, E. Lackner, W. Jäger, M. Höferl, M. Zeitlinger, Lack of dermal penetration of topically applied gentamicin as pharmacokinetic evidence indicating insufficient efficacy, J. Antimicrob. Chemother. 73 (10) (2018 Oct 1) 2823–2829.
[24] M. Brunner, P. Dehghanyar, B. Seigfried, W. Martin, G. Menke, M. Müller, Favourable dermal penetration of diclofenac after administration to the skin using a novel spray gel formulation, Br. J. Clin. Pharmacol. 60 (5) (2005 Nov) 573–577.
[25] S. Miyatake, H. Ichiyama, E. Kondo, K. Yasuda, Randomized clinical comparisons of diclofenac concentration in the soft tissues and blood plasma between topical and oral applications, Br. J. Clin. Pharmacol. 67 (1) (2009 Jan) 125–129.
[26] A. Naik, Y.N. Kalia, R.H. Guy, Transdermal drug delivery: overcoming the skin's barrier function, Pharmaceut. Sci. Technol. Today 3 (9) (2000 Sep 1) 318–326.
[27] G.K. Menon, A.M. Kligman, Barrier functions of human skin: a holistic view, Skin Pharmacol. Physiol. 22 (4) (2009) 178–189.
[28] M.R. Prausnitz, S. Mitragotri, R. Langer, Current status and future potential of transdermal drug delivery, Nat. Rev. Drug Discov. 3 (2) (2004 Feb) 115–124.
[29] B. Azadgoli, R.Y. Baker, Laser applications in surgery, Ann. Transl. Med. 4 (23) (2016 Dec) 452.
[30] R. Langer, Transdermal drug delivery: past progress, current status, and future prospects, Adv. Drug Deliv. Rev. 56 (5) (2004 Mar 27) 557–558.
[31] J.O. Blakeley, P. Wolkenstein, B.C. Widemann, J. Lee, L.Q. Le, R. Jackson, et al., Creating a comprehensive research strategy for cutaneous neurofibromas, Neurology 91 (2 Suppl 1) (2018 Jul 10) S1–S4.
[32] A. Cannon, K. Jarnagin, B. Korf, B.C. Widemann, D. Casey, H.S. Ko, et al., Clinical trial design for cutaneous neurofibromas, Neurology 91 (2 Suppl 1) (2018 Jul 10) S31–S37.
[33] A. Cannon, M.J. Chen, P. Li, K.P. Boyd, A. Theos, D.T. Redden, et al., Cutaneous neurofibromas in Neurofibromatosis type I: a quantitative natural history study, Orphanet J. Rare Dis. 13 (1) (2018 Feb 7) 31.
[34] Y. Li, L. Guo, W. Lu, Laser ablation-enhanced transdermal drug delivery, Photon. Laser Med. 2 (4) (2013) 315–322.
[35] Y.N. Kalia, Y.G. Bachhav, T. Bragagna, C. Bohler, P.L.E.A.S.E.® (Painless Laser Epidermal System). A new laser microporation technology, Drug. Deliv. Technol. 8 (2008) 26–31.
[36] N.K. Brogden, M. Milewski, P. Ghosh, L. Hardi, L.J. Crofford, A.L. Stinchcomb, Diclofenac delays micropore closure following microneedle treatment in human subjects, J. Contr. Release 163 (2) (2012 Oct 28) 220–229.
[37] N. Numan, M. Elmas, Dermoscopy of cutaneous neurofibromas associated with neurofibromatosis type 1, J. Am. Acad. Dermatol. 73 (3) (2015 Sep) 529–531.
[38] P. Gattuso, V.B. Reddy, O. David, D.J. Spitz, M.H. Haber, Differential Diagnosis in Surgical Pathology, Elsevier Health Sciences, 2014, p. 108.
[39] J. Peltonen, R. Penttinen, H. Larjava, H.J. Aho, Collagens in neurofibromas and neurofibroma cell cultures, Ann. N. Y. Acad. Sci. 486 (1986) 260–270.
[40] C.R. Antonescu, B.W. Scheithauer, J. Woodruff, Tumors of the peripheral nervous system, in: Afip Atlas of Tumor Pathology Series 4, 19, 2013.
[41] S. Wang, B. Liechty, S. Patel, J.S. Weber, T.J. Hollmann, M. Snuderl, M.A. Karajannis, Programmed death ligand 1 expression and tumor infiltrating lymphocytes in neurofibromatosis type 1 and 2 associated tumors, J. Neuro Oncol. 138 (1) (2018 May) 183–190.
[42] M. Julião, I. Cunha, A. Costa, Skin necrosis after subcutaneous diclofenac administration: a case report, J. Palliat. Med. 19 (6) (2016 Jun) 584–585.
[43] M. Lai, Y.C. Huang, P.C. Yao, Tissue necrosis after intramuscular administration of diclofenac: report of one case, Acta Paediatr. Taiwanica 46 (1) (2005 Jan-Feb) 24–26.
[44] Y.G. Bachhav, S. Summer, A. Heinrich, T. Bragagna, C. Böhler, Y.N. Kalia, Effect of controlled laser microporation on drug transport kinetics into and across the skin, J. Contr. Release 146 (1) (2010 Aug 17) 31–36.
[45] K. Wiech, M. Ploner, I. Tracey, Neurocognitive aspects of pain perception, Trends Cognit. Sci. 12 (8) (2008 Aug) 306–313.