Effects of a Novel Ophthalmic Solution Containing Glicopro® Complex on Signs and Symptoms of Patients with Dry Eye Disease

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Abstract

Purpose

To evaluate the changes in signs and symptoms of patients with dry eye disease (DED) treated with a novel tear substitute based on the GlicoPro® complex along with its effects on the tear content.

Methods

In this prospective study, patients with DED not successfully responding to conventional tear substitutes were treated with a novel eye drop based on the GlicoPro® complex (posology of 4 times daily). Patients were examined before starting study treatment (T0) and after 30 days (T1) and 60 days (T2) by means of Keratograph 5M (Oculus, Wetzlar, Germany) for the evaluation of: i) tear meniscus height (TMH); ii) non-invasive breakup time (NIBUT) a) first, b) average and c) class; iii) bulbar redness; iv) infrared meibography for the calculation of meibomian glands loss (MGL). Symptom Assessment in Dry Eye (SANDE) questionnaire was administered at each time point to assess ocular discomfort symptoms. In the subgroup of patients whose TMH at T0 was ≥ 0.25 mm, the analysis of tear content was conducted to measure Proenkephalin and Met/Leu-enkephalinproenkephalin (reported as processed active peptides).

Results

Overall, 60 patients (23 males, 37 females; mean age 67.00 ± 8.00 years) were enrolled. At T2, a significant improvement of NIKBUT first (from 4.01 [2.87−5.88] seconds [s] to 7.90 [5.28−11.76] s; $p < 0.0001$), NIKBUT average (from 9.63 ± 5.03 s to 13.85 ± 4.88 s; $p < 0.0001$), NIBUT class (from 1.00 [0.00−2.00] to 1.00 [0.00−1.00]; $p < 0.05$) and TMH (from 0.28 [0.21−0.39] millimetres [mm] to 0.32 [0.24−0.40] mm; $p < 0.01$); in parallel, SANDE score significantly decreased at T2 (from 60.60 [52.21−68.90] to 35.60 [27.53−44.33]; $p < 0.0001$). In the subgroup of patients (n = 9) undergone tear analysis, a statistically significant increase in the mean value of enkephalins and proenkefalin was observed at T2 and T1 respectively (from 1 ± 0.56 to 1.46 ± 1.24; $p < 0.01$ and 1 ± 0.63 to 1.43 ± 0.73; $p < 0.01$). No patients reported adverse events related to study treatment.

Conclusions

The novel tear substitute based on GlicoPro® resulted in significant improvement of ocular discomfort symptoms as well as tear volume and stability in patients with DED not responding to conventional tear substitutes. The increase in active peptides processed in tears may represent the pathophysiological substrate underlying this finding.

1 Introduction

Dry eye disease (DED) is a common multifactorial ocular surface disease characterized by an imbalance in tear film homeostasis and associated with ocular symptoms such as irritation and burning.[1,2] As

highlighted in the definition of the International Dry Eye Workshop (DEWS), inflammation, ocular surface damage, i.e., loss of conjunctival goblet cells and increased squamous metaplasia of surface epithelial cells, and neurosensory abnormalities play an important etiologic role.[3,4] Evaporation of the aqueous component of the tear film and the resulting instability lead to a state of tear hyperosmolarity, which is one of the central mechanisms of DED. Hyperosmolarity can trigger the activation of the inflammatory cascade that leads to cellular damage, particularly through the loss of conjunctival cells that produce mucin, which further exacerbates tear film instability and creates a vicious cycle.[5–7] Lam et al. showed in patients with DED that interleukin (IL) -6, IL -8, and tumor necrosis factor (TNF)-alfa were significantly increased compared with controls. In particular, IL -6 and irritation symptom severity were significantly correlated, suggesting that IL -6 may be the result of neuropathic eye pain.[8] Patient-reported symptoms include burning, foreign body sensation, and ocular discomfort, which include dryness, irritation, grinding, scratching, soreness, stinging, burning, itching, and eye fatigue to pain, compromising quality of life and work productivity, and thus posing a serious public health problem.[9–12]

Current treatments can only partially ameliorate symptoms, and there is the continuous need for therapies working on novel targets. Recently, a new therapeutic approach for the control of discomfort/pain symptoms involving opiorphin, an endogenous peptide with potent analgesic properties due to its ability to enhance endogenous opioid signaling by protecting enkephalins from degradation, has been proposed.[13] Opiorphin is one of the major endogenous metabolites and is also secreted in tears and its concentration increases in response to pain.[14] Unlike topical anesthetics, opiorphin does not determine an obstacle to the repair of the epithelial defect, alteration of lacrimation, increased corneal permeability with edema and opacification, and, finally, alteration of the elements of the cytoskeleton of the corneal epithelium with alteration of cell motility.[15] Furthermore, it has a lower ability to induce tolerance, and dependence compared to the direct opioid agonist morphine. In fact, evidence suggests that after 7 days of treatment, the analgesic effect of opiorphin exceeds the one induced by morphine at a similar dose, demonstrating the progressive loss of efficacy of the latter and the maintenance of the effect over time of the former.[16,17]

A new ophthalmic solution (Lacricomplex®, FB Vision, Ascoli Piceno, Italy) containing GlicoPro®, a multimolecular complex based on proteins and sulfured and unsulfured glycosaminoglycans (GAGs) - useful for lubricating, stabilizing tear film and prolonging pre-corneal persistence - and opiorphin, which assists the physiological pain-relieving mechanism of the eye by enhancing Met- and Leu-enkephalins concentrations, has become recently commercially available.[18–21] In DED corneal tissues treated with GlicoPro histomorphological analysis demonstrated restoration of the corneal epithelium, microvilli, and mucin network.[18]

The purpose of this study was to evaluate the changes of subjective symptoms and objective signs occurring in patients suffering from DED, not responding to conventional tear substitutes, treated with Lacricomplex®; the effects of treatment on the tear concentration of enkephalins and precursor proenkephalin was also investigated.

2 Methods

Study and Patients

In this prospective study, patients with DED were examined and screened for enrolment at a tertiary referral center (Department of Ophthalmology, University of Magna Grecia, Catanzaro, Italy) between March 2022 and December 2022.

The study was approved by the local Ethics Committee (Comitato Etico Regione Calabria – Sezione Area Centro, protocol n. 239–2022). A detailed informed consent for participation in the study was signed by all patients, in accordance with the 1964 Declaration of Helsinki.

Inclusion criterion was the diagnosis of DED not successfully controlled with conventional tear substitutes. Patients were excluded if one or more of the following conditions were present: recent (within 3 months) ocular surgery, systemic disease or therapies affecting tear secretion, concomitant ocular diseases or use of other topical medications (e.g., corticosteroids, NSAIDs). Baseline demographic and clinical characteristics of enrolled patients are summarized in Table 1.

Table 1
Baseline demographic and clinical patient's characteristics

Age, mean value (SD), years	67.00 (8.00)
Sex (M/F)	23/37
Caucasian race, N (%)	60 (100)
TMH, median value (IQR), mm	0.28 (0.21−0.39)
NIKBUT first, median value (IQR), s	4.01 (2.87−5.88)
NIKBUT average, mean value (SD), s	9.63 (5.03)
SANDE score, median value (IQR)	60.60 (52.21−68.90)
Bulbar redness score, median value (IQR)	1.35 (1.02−1.60)
MGL scale, median value (IQR)	1.50 (1.00−2.00)
Abbreviations: SD, standard deviation; IQR, interquartile range; TMH, tear meniscus height; NIKBUT, non-invasive breakup time; SANDE, Symptom Assessment in Dry Eye; MGL, meibomian glands loss; mm, millimeters; s, seconds.	

Patients who satisfied study criteria were enrolled and treated with Lacricomplex® according to the following therapeutic regimen: 2 drops 4 times daily for 60 days in both eyes.

Ocular Surface Workup

All patients underwent non-invasive examination of the ocular surface using Keratograph 5M (Oculus, Wetzlar, Germany), before starting treatment (T0) and 30 ± 2 days (T1) and 60 ± 4 days (T2) after, for the

evaluation of: i) tear meniscus height (TMH); ii) non-invasive break-up time (NIBUT) a) first, b) average, c) class (0:>10 seconds [s]; I: 6–10 s; II 3–6 s; III < 3 s); iii) bulbar redness; iv) infrared meibography for evaluating meibomian glands loss (MGL). Symptoms of ocular discomfort were assessed using the Symptom Assessment in Dry Eye (SANDE) questionnaire.

Tear Analysis

In a subgroup of patients with adequate amount of tears (TMH value $\geq$ 0.25 millimetres [mm] at T0), a sample of 10 µL of tears was collected to evaluate the concentration of Proenkephalin and Met/Leu-enkephalinproenkephalin. Briefly, tears collected by capillary tube were blown into microcentrifuge tubes, stored at − 80°C, and used within 2 months. Total protein amount in the tears were determined by performing a Bradford Assay according to the manufacturer's recommendations (Protein assay Dye reagent concentrate, Bio-Rad, Hercules, US-CA). The absorbance was measured at 595 nm with a spectrophotometer (NanoDrop One, Thermo Scientific™) and the protein concentrations were de-rived using a bovine serum albumin (BSA) calibration curve. Thirty-five micrograms of tear proteins were used for Western Blot analyses and 10 micrograms for Coomassie Blue staining. Separation by SDS-PAGE, blotting, and incubation with primary and secondary antibodies were performed as de-scribed elsewhere.[22] Chemiluminescence reaction was carried out with Clarity Western ECL Substrate (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and the signal acquisition was performed through the ChemiDoc Xrs + by the Image Lab software (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Antisera and monoclonal antibodies used in the present work are the following: HRP-conjugated anti-mouse (Sigma Aldrich, Saint Louis, MO, USA) and anti-human Met/Leu-enkephalin (cat. No. sc-47705; Santa Cruz Biotechnology, Santa Cruz, CA, USA). In the Coomassie Blue staining following SDS-PAGE the polyacrylamide gel was stained with a staining solution (0.4% Coomassie Blue, 50% methanol, 10% acetic acid) for 30 min at room temperature. The gel was sequentially soaked into a destaining solution-I (50% methanol, 10% acetic acid) for 30–60 min until the protein bands were dis-cretely observable. Signals were analyzed by ImageJ software.

Outcomes

The primary outcome was the changes of objective signs and subjective symptoms occurring after the study treatment. The secondary outcome was the change of tears content (Proenkephalin and Met/Leu-enkephalinproenkephalin) registered after treatment in a subgroup of patients with adequate amount of tears.

Statistical Analysis

Statistical analyses were performed Prism version 9.4.0 (GraphPad Software Inc., San Diego, CA, USA). Normally distributed data were expressed as mean ± standard deviation (SD), otherwise as median values with interquartile range (IQR). Parametric and nonparametric tests were chosen on the basis of data normality. The Anderson-Darling test and Kolmogorov-Smirnov tests were applied to assess if data were normally distributed. Student t test, Mann-Whitney U test, Dunnett's multiple comparison test and

Friedman test were used to compare variables, when appropriate. A p-value less than 0.05 was considered statistically significant. To determine the sample size of the study, a priori power analysis was performed based on the data of the study of Lambiase et al. In total, 19 patients were required to detect a mean change of SANDE from baseline of 16.1 points, with a power of 0.95 and a P value of 0.05.[23]

3 Results

A total of 60 patients (23 males, 37 females; mean age 67.00 ± 8.00 years) with DED were included in the study. At both T1 and T2, a significant improvement of NIKBUT first (from 4.01 [2.87−5.88] s to 6.89 [4.01−8.98] s [p = 0.0001] and 7.90 [5.28−11.76] s [p < 0.0001], respectively) and NIKBUT average (from 9.63 ± 5.03 s to 11.72 ± 3.84 s; p < 0.01 and 13.85 ± 4.88 s; p < 0.0001, respectively) was found; NIBUT class showed a significant improvement at T2 (from 1.00 [0.00−2.00] to 1.00 [0.00−1.00] [p < 0.05]). (Fig. 1).

The mean value of TMH increased significantly from T0 to both T1 and T2 (from 0.28 [0.21−0.39] mm at T0 to 0.31 [0.27−0.40] [p < 0.05] and 0.32 [0.24−0.40] [p < 0.01], respectively). SANDE score significantly decreased from a baseline value of 60.60 (52.21−68.90) to 43.72 (39.00-50.98) (p < 0.0001) at T1 and 35.60 (27.53−44.33) (p < 0.0001) at T2. (Fig. 2)

Conversely, no statistically significant reduction was detected at each time point for bulbar redness (from 1.35 [1.02−1.60] at T0 to 1.30 [1.00-1.77] and 1.20 [1.00-1.60] [p > 0.99], respectively) and MGL (from 1.50 [1.00−2.00] at T0 to 2.00 [1.00−2.00] and 2.00 [1.00−2.00] [p > 0.99], respectively).

In the subgroup of patients in which tear analysis was conducted (n = 9), a statistically significant increase was found at T2 for the processed active peptides (from 1 ± 0.56 to 1.53 ± 0.76; p < 0.01]) and ar T1 for proenkephalin (from 1 ± 0.63 to 1.43 ± 0.73; p < 0.01]) (Fig. 3). No adverse events related to the use of eye drop were reported during the entire study.

4 Discussion

The present study reports the preliminary results of the first clinical experience with a novel ophthalmic preparation based on GlicoPro®, a multimolecular complex with lubricating, moisturizing, antioxidant and protective effects. After 2 months of treatment, NIKBUT (both first and average) and TMH improved significantly at each time point; in parallel, ocular discomfort symptoms evaluated by SANDE score reduced significantly. Clinical outcomes were further supported by molecular changes detected in the tear fluid of patients who received the treatment. In fact, a significant increase in the tear content of proenkephalin and processed active peptides was detected respectively after 1 month and 2 months of therapy. A previous in vitro study showed that the product contributes to the physiological repair processes at the corneal level, supporting the restoration of the functionality of the corneal nerve terminations and the process of corneal healing with a significant inhibitory effect on enkephalinase enzymes.[11] In the present study, this effect was also confirmed by the increase in the tear fluid of patients

of Met- and Leu-enkephalin and, interestingly, of the precursor proenkephalin. This effect is mainly ascribed to opiorphin, a natural peptide contained in the protein fraction of GlicoPro®, which assists the physiological pain-relieving mechanism of the eye. Opiorphin is endogenously present in body fluids, primarily in tears, and its level is enhanced in pathological, painful conditions. Salivary opiorphin is increased in patients with Burning Mouth Syndrome,[24] as well as in patients with dental pain caused by pulp inflammation.[25] In tears, opiorphin levels increase in the presence of ocular pain caused by a corneal foreign body.[26] The role of opiorphin in pain modulation has been extensively described;[26–29] this pentapeptide enhances the endogenous opioid signaling by protecting enkephalins metabolism. Opiorphin is an inhibitor of the enzymes neprilysin (neutral endopeptidase) and aminopeptidase N, consequently the Met-enkephalin and Leu-enkephalin concentration increases.[28,30,31] The pharmacodynamic peculiarity makes opiorphin a physiological pain modulator able to counteract nociceptive and neuropathic pain reducing hypersensitivity with an opioid receptor-dependent mechanism.[32,33] The safety profile of opiorphin is significantly better than direct opioid agonists since its effects depend on the concentration of enkephalins released in response to a painful stimulus rather than a direct receptor action.[34]

The observed enhanced concentration of Leu- and Met-enkephalin by about 50% suggests the relevance of the opioid signaling in the relief of ocular discomfort. It should also be noted that, the repeated treatment with GlicoPro® increased the enkephalin precursor proenkephalin in tears at T1 but not overall. This could confirm that the opiorphine-induced modulation of opioid signaling could be mainly due to its catabolism-reduction activity rather than anabolism-enhancement of enkephalin.

The present study suffers from a limitation that should be mentioned. The study design lacks a control group of patients treated with placebo or vehicle. However, we included patients who were not satisfactorily controlled with other tear substitutes; thanks to the switch to new formulation, patients were able to better manage ocular discomfort symptoms as showed also by improvement of tear film stability and volume. The study was not powered to detect differences in tear content thus the lack of significance of proenkephalin variation at T2 could be related to the small sample size of the subgroup of patients with an adequate tear volume required to collect tears avoiding reflex tearing.

In conclusion, topical treatment with a novel GlicoPro®-based tear substitute significantly improved ocular discomfort symptoms and objective signs in patients who did not respond to conventional tear substitutes. These findings suggest a special ability of the new product thanks to the multi-target approach allowed by its complex composition.

Declarations

Author Contributions

GG, GCS, VS, LDCM, RS, CG: conception, design, writing, review, and revision pf the manuscript. SV, MB, GS, GCS acquisition of data (patients follow up and examination), analysis and interpretation of data. LZ, IP, PV, TZ development of methodology, analysis and interpretation of data, writing, review, and revision of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to privacy or ethical restrictions but are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Informed consent was acquired from all the participants, and the study was carried out in accordance with the Declaration of Helsinki of 1964 and its later amendments, with approval from the local institutional ethics committee (Comitato etico Regione Calabria, protocol number 239-2022).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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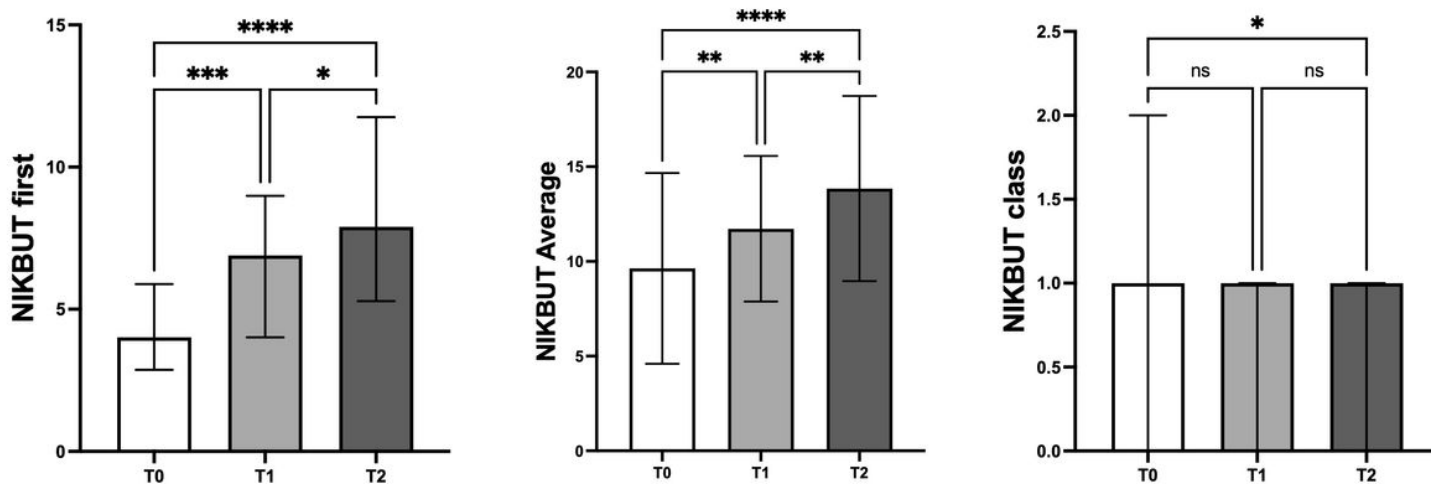
Figures

Figure 1

Values of NIKBUT first, average and NIBUT class at each time point. Ns = not significant; * p < 0.05; ** p < 0.01; *** p < 0.001, **** p < 0.0001.
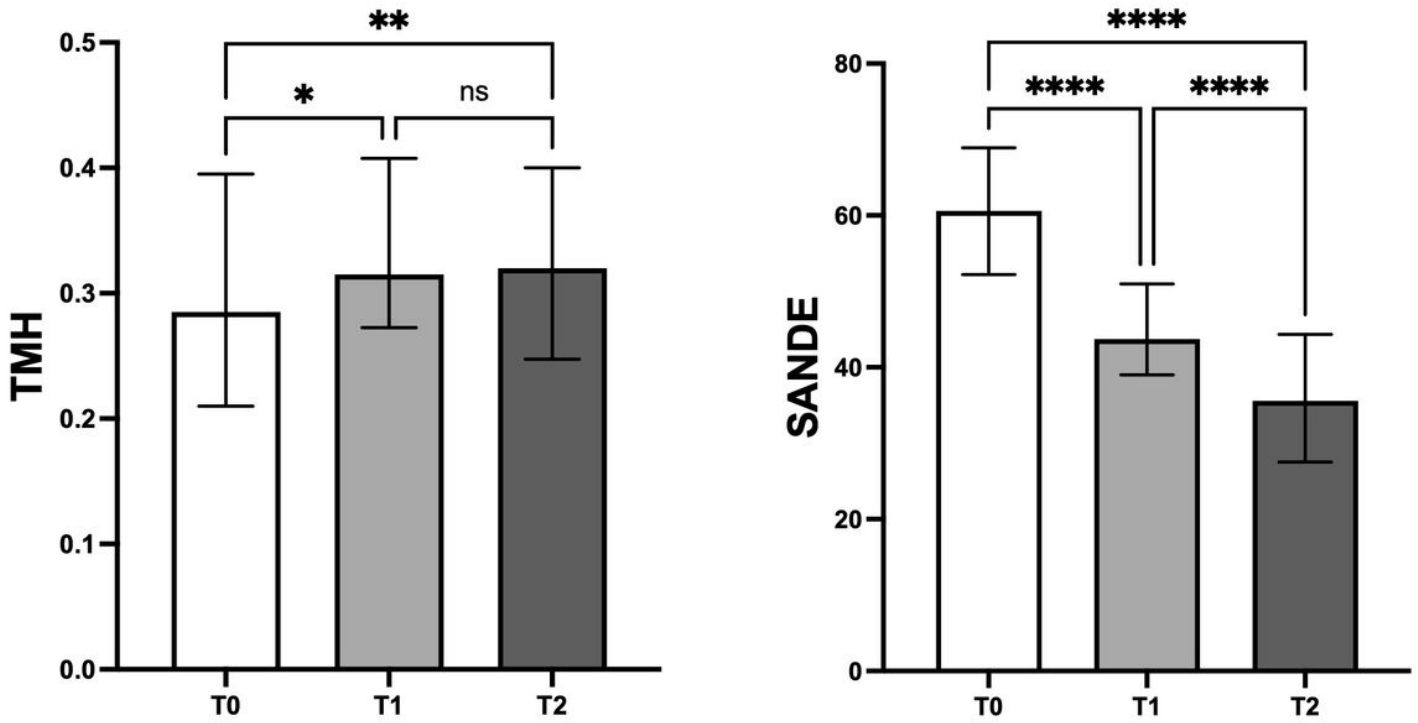
Figure 2

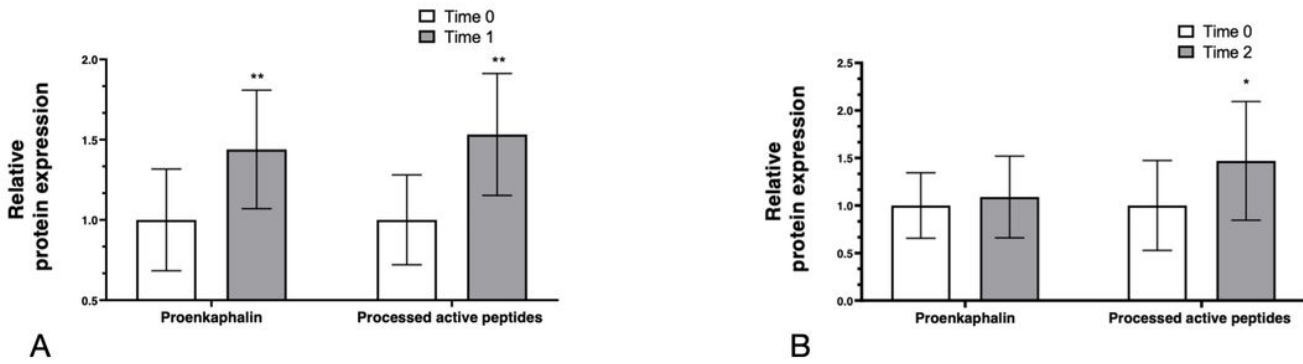Values of TMH and SANDE score at each time point. * p < 0.05; **p < 0.01; **** p < 0.0001.

Figure 3

(A) Proenkephalin and Met/Leu-enkephalin (reported as processed active peptides) levels at T0 and T1. (B) Proenkephalin and enkephalin levels at T0 and T2. Statistical analysis was performed with t test (* p < 0.05; ** p < 0.01).