ENKORTEN®

6 mg

Metenkolin acetate 5 mg
Tridecains acetate 1 mg

6 ampulla + 6 inotrofe osnove za inotropiranje

FARMACIJA d.o.o.
Tuzla
ENKORTEN®

A novel non-interferon drug for Multiple Sclerosis treatment and other inflammatory and immunomodulatory diseases utilizing two neuropeptides that participate in modulation of immunological processes.

Farmacija d.o.o. from Tuzla, Bosnia and Herzegovina has developed a new promising treatment for Relapse Remitting form of Multiple Sclerosis that is used as a disease modifying treatment and at the same time as a treatment during the relapse of MS, substituting the corticosteroid pulse therapy. As a combination of two endogenous neuropeptide components, Enkorten modulate immunological processes with analgesia, antipyretic, antioxidant and anti-inflammatory effects without side effects related to steroid and non-steroid anti-inflammatory drugs. Pharmacodynamic effects rely directly on basic mechanisms of development of immunologic response.

Other indications in development:

- Crohn’s Disease
- Ulcerative Colitis
- Rheumatoid Arthritis
- Asthma
- Psoriasis

Composition
1 vial lyophilisate:
  • metenkefalin acetate: 5 mg
  • tridecactide acetate: 1 mg

Solvent: 0.9% NaCl in 2 ml ampoule

Pharmaceutical form
Lyophilised powder + solvent for solution for injection

Production
The product quality is ensured through outsourcing of the production process and control of active ingredients and final production and control. Manufacturing of both active substances is conducted in the GMP compliant facilities by PPL, USA. BAG Health Care, Lich, Germany is a manufacturer of the lyophilised product in accordance to GMP requirements. Testing of Enkorten® for release is performed by BAG Health Care. Secondary packaging and batch release is conducted by Farmacija d.o.o. Tuzla at moment; however, production and packaging are easily transferable and feasible at low cost to targeted markets.
**Development status**

Full registration for indication of relapse remitting multiple sclerosis in Bosnia and Herzegovina issued in February 2010.

Conditional Regulatory Approval in Federation of Bosnia and Herzegovina from August 2006 until August 2008.

**Pharmacological properties**

ATC code: L03AX

**Pharmacodynamics**

ENKORTEN is a combination of two peptide components which have endogenous origin: metenkefalin and tridecactide (with an identical 13 amino acids sequence as α-melanocyte stimulating hormone (α-MSH), but without acetyl and amid end). Both peptides belong to the group of neuropeptides. Both neuropeptides participate in modulation of immunological processes. Metenkefalin and tridecactide exert cytoprotective effect in an animal model of ethanol induced gastric lesion in rats when applied individually, however a statistically significant additive effect is shown when a combination of both components is applied. Effect of both peptides includes analgesia, antipyretic and antioxidative effect as well as anti-inflammatory activities without most of the side effect of steroid and non-steroid anti-inflammatory drugs.

Method of selective immunomodulation via application of peptides has recently been used in immuno-mediated diseases and malignant neoplasma. Immunomodulation based on antigens and peptides is targeted toward achieving long term satisfying remission without appearance of toxic side effects characteristic of immunosuppressive drugs. It is based on two approaches: first approach is blocking of initial activation of antigen recognition of autoreactive T cells, and second one is based on downregulation of specific antigenic inflammatory response of T cells through activation of regulatory physiological pathways.

Immunomodulatory effects of the combination of peptides in Enkorten rely directly on basic mechanism of development of immunologic response. Modification of cytokines production can be emphasized as one of the immune response modulation. Their production causes activation of leukocytes and further release of their mediators.

Wide spectrum of anti-inflammatory activities of tridecactide suggests inhibition of critical step that is common in different forms of inflammation. Three basic mechanisms of anti-inflammatory action could be selected: direct effect on melanocortin receptors on cells on periphery (monocyte/macrophage and neutrophiles), action on glia receptors and downregulation of anti-inflammatory pathways through melanocortin receptors on neuronal cells. It has been shown that α-MSH inhibits activation of nuclear transcription factor NK-κB, which activates TNFα.

Enkephalines act in nervous system through opioid receptors. Met-enkephalin has high affinity for the OP1 (δ) receptors. Normal human T-lymphocytes, granulocytes, complements, thrombocyte and B lymphocytes possess receptors for met-enkephalin.

**Pharmacokinetics**

After subcutaneous application of Enkorten, the concentration/time curve for met-enkephalin points at the kinetics of first order. Area under the concentration/time curve from t2 to t∞ (AUCinf) is 360.64 pg/h/ml. Maximum measured concentration of met-enkephalin in serum Cmax 1266.14 pg/ml, time to reach the maximum concentration in blood after drug administration is Tmax 0.16 sati. Based on pharmacokinetic studies after subcutaneous application, met-enkephalin showed a short plasmatic half-life, although as an endogenous
substance it can have a longer biological half-life. The average value of half-life of met-enkephalin is 0.24 hours (14.4 minutes) and ranges between 4.2 and 39 minutes. The kit for determination of ACTH 1-13 as a component of Enkorten is not available. Therefore, the pharmacokinetic study on this substance is not performed. Considering that ACTH 1-13 and α-MSH have an identical amino acids sequence, cross-reactivity of these two peptides was determined. Results showed that ACTH 1-13 in Enkorten does not influence plasma values of endogenous α-MSH.

Active substance from Enkorten do not absorb from gastrointestinal tract. As peptide, they undergo denaturation and decomposition in gastrointestinal tract. They are applied parenterally. Indirect data from toxicology studies suggest that drug metabolism occur in liver.

**Preclinical safety study**

**Single dose toxicity**

No lethal outcomes were registered during the studies. Therefore, it was not possible to determine LD₅₀ (lethal dose 50%) for Enkorten. Three separate studies of the acute toxicity were conducted on rats: intravenous application according to modified method of acute class toxicity study, (Acute class toxicity method, ATC), subcutaneous application study and intraperitoneal application with determined limit dose method. The highest intravenous applied dose was 200 times, the highest subcutaneous dose was 500 times higher and the highest intraperitoneal dose was 1000 times higher than the supposed human therapeutic dose. In the intravenous application study, mild decrease of motor activity in all animals in group treated with highest doses was registered and also in all male animals treated with middle dose. In female animals in this group mild increase in motor activity was observed. Horizontally positioned tail is noticed in all animals treated with highest dose. The mild cyanosis and vasoconstriction in one male from group treated with highest and middle dose was observed. Statistically significant differences in reaction to touch in males of all experimental group were noticed after intravenous and subcutaneous application of Enkorten. Also significant differences between females treated with highest dose compared to control group were registered. In the intraperitoneal application study a significant clinical changes of a group treated with 1000 times higher dose than the supposed human therapeutic dose were registered. In all treated males during the first day after the application an irregular breathing, lower motor activity, somnolence, ataxia, catalepsy and muscle hypotonia were noticed. No significant disorders of character and frequency of breathing were noticed in all three studies. Clinical signs registered during the study were mostly of reversible nature. Also in all acute toxicity studies no macroscopic and microscopic changes were noticed. There were no changes of weight of single organs after sacrifice at the end of the study.

**Toxicity of repeated dose**

The subacute toxicity study on rats was conducted. Three dose levels were determined for subcutaneous application: approximate maximal human dose (0.143 mg/kg metenkefalin + 0.028 mg/kg tridecactide), 5 times higher dose and 10 times higher dose. In studies of subacute toxicity no lethal outcome was registered. Maximum tolerated dose for males was approximately 5 times higher dose than supposed maximum human therapeutic dose (significant difference was registered in biochemical parameters with higher dose level) and for females maximum tolerated dose corresponded to the lowest dose implemented in the study (significant difference was registered in values of ALAT in higher dose levels). The study of subchronic toxicity was conducted on rats. Three dose levels were determined for intramuscular application. Approximate human dose (0.071 mg/kg metenkefalin + 0.014 mg/kg tridecactide), 5 times higher dose and 10 times higher dose. No lethal outcomes were registered. Maximum tolerated dose for males and females correspond to 10 times higher dose
of supposed human therapeutic dose. The study of chronic toxicity was conducted on rats in three dose levels for subcutaneous application: approximate maximum human dose, 5 times and 10 times higher maximum human dose. In studies of chronic toxicity no lethal outcome was registered. Statistically significant differences in sensitivity to pain stimulus and frequency of vocalisation to touch were noticed between experimental and control group of animals. In all toxicity studies no lethal outcome was registered. Also, Enkorten did not influence macroscopic and microscopic structure of organs and tissues in all experimental animals. Changes in hematological and biochemical parameters that were registered during toxicity studies were inside referential values. Two studies of safety pharmacology were made: after a one single dose subcutaneous application of the substance and after a one single dose intraperitoneal application of the drug. No effect of applied substance on monitored parameters of cardiovascular and respiratory function on CNS was registered.

**CLINICAL:**

**Clinical phase III - MS**

Randomised, prospective, comparative clinical study phase III on 80 patients was conducted at Clinic for neurology, University clinical centre of Sarajevo. Number of relapses, relapse free time, size, volume and number of T1 and T2 lesions measured by MRI, EDSS score as a measure of disability and safety of Enkorten were evaluated.

In the experimental group a significant decrease in the EDSS were noted, whereas statistically significant increase in EDSS was noted within the control group. A reduction of 63% in number of relapses was seen in the experimental group compared to the control group during the study. In addition, duration of relapse state during the study was 67% shorter in experimental compared to control group. Cumulative probability of time to first relapse was statistically longer in patients taking Enkorten (experimental group) compared to patients who were free from medication for MS (control group). 85% of patients in experimental and 66.7% in the control group remained relapse free throughout the study. During the relapse patients in the experimental group received 12 mg of Enkorten for three consecutive days instead of corticosteroid pulse therapy.

Statistically significant decrease in average diameter of T1 lesions and average diameter, maximum diameter and volume of T2 lesions was noted within the experimental group during the study. Comparing control and experimental groups showed a good statistical trend towards reduced maximum diameter, volume and number of T1 and T2 lesions measured at the end of the study in experimental group in patients who were taking Enkorten, compared to ones who were free from medication for MS.

Strong safety profile that was seen in phase II clinical study was maintained in the phase III clinical study. Again, there was no serious and new adverse events reported.
**CLINICAL STUDIES CONCLUSIONS**

**Number of relapses:** Statistically significant reduction in number of relapses was noted in the experimental group compared to control group during the studies.

**Duration of relapse:** In addition, duration of relapse state during the study was significantly reduced in experimental compared to control group.

**Time to first relapse:** Cumulative probability of time to first relapse was statistically longer in patients taking Enkorten (experimental group) compared to patients who were free from medication for MS (control group).

**Number of relapse-free patient:** 72% of patients in experimental and 42.3% in the control group remained relapse free throughout the clinical phase II. 85% of patients in experimental and 66.7% in the control group remained relapse free throughout the clinical phase III.

**Enkorten** was used as a treatment for relapses during the study in the patients from the experimental group. Enkorten has demonstrated its ability as a substitution for corticosteroid pulse therapy during the relapses with significant reduction in duration of these relapses and absence of adverse events that usually accompany corticosteroids. 100% of patients that had relapses of the MS in the experimental group were free from corticosteroids during 12 months of the study.

**Enkorten** has shown remarkable safety profile with no severe adverse events being reported during 1 year of Enkorten application.
**Patent information**

Farmacija Tuzla has the exclusive world-wide license to Enkorten.

US Patent No. 7544653 issued on June 09, 2009. Title: Additive cytoprotective effects of two bioactive regions of pro-opiomelanocortin hormone

Australian patent No. 2002240733 issued on March 17, 2009. Title: Additive cytoprotective effects of two bioactive regions of pro-opiomelanocortin hormone

Granted Russia Patent No. 2297238 on April 20, 2007. Title: Additive cytoprotective effects of two bioactive regions of pro-opiomelanocortin hormone

Granted China Patent No. ZL01823710.X, Grant Announcement Date June 20, 2007, Title: Additive cytoprotective effects of two bioactive regions of pro-opiomelanocortin hormone

Pending EU Patent application No. 01988010.3. Title: Additive cytoprotective effects of two bioactive regions of pro-opiomelanocortin hormone

Other international filings are beyond filing stage and include: International application published by the World Intellectual Property Organisation under number: WO 03/033017

**Publications and Congress Presentations**


Kusturica J, Mulabegović N, Bečić F, Mandal S, Todić M, Kapić E, Loga S, Plasma methionine-enkephalin and alpha melanocyte-stimulating hormone concentration after the subcutaneous application in rats. Division of Clinical Pharmacology and 7th Congress of the European Association for Clinical Pharmacology and Therapeutics (EACPT), Poznan, Poland, 25-29 June, 2005


**PhD and MA Dissertations:**

Maida Todic: Toksikološki modeli ispitivanja akutne toksičnosti peptida. Magistarski rad, Medical Faculty, University of Sarajevo, 2004

Dorian Tješić-Drinković: Utjecaj met-enkefalina i alfa-hormona koji stimulira melanocite na plućnu funkciju u eksperimentalnom modelu astme zamorca, PhD Dissertation, Medical Faculty, University of Zagreb, 2002

Duška Tješić-Drinković: Utjecaj alfa-MSH i met-enkefalina na eksperimentalni colitis štakora, PhD Dissertation, Medical Faculty, University of Zagreb, 2002

Fahir Bečić: Ispitivanje hronične toksičnosti mješavine met-enkefalina i alfamelanostimulirajućeg hormona, PhD Dissertation, Medical Faculty, University of Sarajevo, 2006

Jasna Kusturica: Odnos plazmatske koncentracije alfa-melanostimulirajućeg hormona i metionin-enkefalina i vrijednosti osnovnih fizioloških i biohemijskih parametara, PhD Dissertation, Medical Faculty, University of Sarajevo, 2008

Maida Todić, Citogenetičko ispitivanje efekata adrenokortikotropnog hormona 1-13 i metenkefalina, PhD Dissertation, Medical Faculty, University of Sarajevo, 2010