SUMMARY

DOCTORAL THESIS

SCIENTIFIC RESEARCH ON PHARMACOKINETICS OF TOPICAL ANTIINFLAMMATORY FORMS

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KEYWORDS: Meloxicam, AINS, transdermal diffusion, penetration enhancers.
INTRODUCTION

Currently a growing interest has been granted to the antiinflammatory medication. Because of its multiple side effects, the oral way of administration of the nonsteroidal antiinflammatory drugs (NSAID’s) is trying to be replaced with a different route. Most of the times, for treating rheumatic diseases a local, topical administration of NSAIDs is preferred. From the very broad issue of NSAIDs, this PhD thesis is focused on studying the pharmacokinetics of NSAIDs.

This paper is a response to the need concerning the formulation of topical anti-inflammatory preparations, seen by studying the literature. This area is continually sought and still has many unexplored issues.

II. THEORETICAL PART – STATE OF KNOWLEDGE

The theoretical part is divided into five chapters. In the first chapter, the current data on the skin and route of administration of semisolid dosage forms are presented. The second chapter of the theoretical part, provides information from the literature concerning the topical administration of drugs, focusing on the methods and the factors that control the dermal and transdermal diffusion. In the third chapter, the emphasis was placed on the current data found in the literature about the NSAIDs, reserving an entire chapter to the representative of the class - Meloxicam – a nonsteroidal inflammatory COX2 inhibitor. The last chapter was focused on the presentation of the theoretical methods used in the quality control of ointments, as representatives of semisolid dosage forms.

III. SPECIAL PART - PERSONAL RESEARCH

III.1. AIM OF THE STUDY

The number of studies concerning the pain has significantly increased in the last decade in the entire world, which shows that this area still has many untapped resources. Going forward, I noticed that there is no topical formulation having Meloxicam as an active substance.

The main interest of this thesis was obtaining genuine and stable semisolid formulations containing as an active substance, a COX2 selective NSAID (Meloxicam), formulated in order to have an optimum dermatopharmacokinetic profile.

III.2. EXPERIMENTAL DESIGN

Experiments carried out within this thesis were conducted in several steps, aimed as:

1. finding the agents that increase the solubility of Meloxicam, a substance with a very small degree of dissolution in usual solvents such as water, phosphate buffers, citrate, borate, lipophilic solvents;

2. to include the Meloxicam solutions prepared in step 1, in some hydrophilic and hydrophobic semi-solid formulations, while maintaining a molecular dispersion;

3. to control the prepared formulations from the organoleptic, viscosity and plasticity point of view;
4. to obtain the Meloxicam standards and to draw the calibration curve for the spectrophotometrical qualitative and quantitative determination;

5. the pharmacokinetic studies which were carried out consisted in determining the in vitro pharmacokinetic profile (transfer and diffusion) of Meloxicam through:
   - artificial membrane;
   - animal skin;

6. to realize comparative studies of the in vitro pharmacokinetics of Meloxicam at physiological pH (7.5) and acid pH (4.5), using the same membranes: artificial and animal;

7. to realize in vivo studies using animal experiments.

III.3. STUDY NO. I – PERSONAL CONTRIBUTIONS TO THE PREPARATION AND TESTING OF THE SEMISOLID DOSAGE FORMS CONTAINING MELOXICAM USED FOR TOPICAL APPLICATION. METHODS TO OPTIMIZE THE DEGREE OF SOLUBILIZATION OF MELOXICAM

III.3.1. AIMS

In this study, one of the objectives was to prepare hydrophilic and lipophilic ointments, with a 0.3% concentration of the active ingredient - Meloxicam.

The incorporation of Meloxicam was investigated using various agents to increase its solubility in hydrophilic and lipophilic semisolid formulations.

III.3.2. MATERIAL AND METHOD

The substances and solutions that were used were: Meloxicam (Unichem Laboratories Ltd.), Carbopol 990 (TM Medchim), methylcellulose (Medchim TM), lanolin (Medchim TM), Grease (Medchim TM), Tween 80 (Merck), triethanolamine (Fluka Chemie AG ), Polyethylene glycol 400 (Merck), Polyethylene glycol 4000 (Merck), glycerol (SC Chimreactiv LLC), sodium hydroxide (Merck), Fenosept (Medchim TM), 0.9% w/v saline solution (B Braun), nipagin (Medchim TM), nipasol (Medchim TM).

The equipment and utensils that were used were: pharmaceutical mortars, electronic scales, electronic pipette 1-5 ml, 1 - 20 ml syringe, Thermo Scientific HAAKE viscotester, Ojeda - Arbussa device tester for determining the plasticity, Consort pH-meter. The obtained ointments were packed in tightly closed polyethylene boxes, and stored at less than 25°C. For taking photographic images, the ointments were transferred into 20ml capacity syringes.

III.3.3. RESULTS

Nine formulations with a concentration of 0.3% Meloxicam were obtained: 7 gels: G1, G2P1, G2P2, G3, G4, G5, G6 and 2 ointments U1 and U2.

The formulations G1, G2P2 and G4 containing 0.3% Meloxicam were translucent, free of particulate matter and of good consistency. In contrast, the remaining formulations containing Meloxicam were presented in the form of suspension-type ointments of a lower consistency, in which the particles in suspension could be seen.
The extension curve shows that the addition of Meloxicam, the scope is in a slightly larger area (p = 4.83191E-07 <0.05).

Of all the formulations, the lowest extension surface was observed for the hydrophilic ointment U2, and the largest, for the G2P2 formula, which shows the importance of excipients in the formulation of semisolid dosage forms.

**III.3.4. DISCUSSIONS**

From the organoleptic point of view, the ointments had the appearance of the characteristic components. For the formulas that contained as active substance Meloxicam, the aspect was of a more intense yellowish color than for those without the active substance. Following analysis, the preparation obtained in this thesis had the pH in the range required by the Xth Romanian Pharmacopoeia.

Analyzing the viscosity values of all preparations, it can be concluded that the addition of small amounts of Meloxicam (0.3%) leads to a slight decrease in viscosity (p = 0.134034).

**III.3.5. CONCLUSIONS**

Following this study, several conclusions can be drawn:

1. In the absence of the carefully chosen increasing solubility agents, Meloxicam cannot be formulated in the form of hydrophilic or lipophilic semisolid preparations for topical application;

2. After numerous tests, the 0.1% Tween 80, 30% Triethanolamine and 3%Polyethylene glycol 400 had the ability to increase the solubility of Meloxicam. These were the only one from all that were tested, that added to the ointment with Meloxicam led to its solubilization, and so, obtaining transparent solution-type ointments,

3. Amongst the suspected agents that increase the solubility, sodium octilsulfate, a surfactant with humectants properties that decrease the liquid - solid surface tension, failed to solubilize Meloxicam.

**III.4. STUDY NO. II - THE PHARMACOKINETIC PROFILE OF IN VITRO RELEASE OF MELOXICAM THROUGH ARTIFICIAL MEMBRANE FROM HYDROPHILIC SEMISOLID FORMULATIONS. INFLUENCE OF THE ABSORPTION PROMOTERS AND OF THE pH**

**III.4.1. AIMS**

The study took into account the observation of the pharmacokinetic profile of Meloxicam by determining its concentration in the receiving solution after application on artificial cellulose membrane using Franz diffusion cell.

**III.4.2. MATERIAL AND METHOD**

The same substances that have been used in the previous study were used in this one also. In addition, the Hanson Research vertical diffusion cells were used with a capacity of 7ml 58-001-455, a 15 mm hole diffusion and a Helix stirrer. The absorbance was read using a Jasco V530 UV-Vis...
Spectophotometer. Teknokroma cellulose membranes of 3.14cm$^2$ surface a pore size of 0.45µm were used.

### III.4.3. RESULTS

The standard solutions were obtained by dissolving 0.01g of Meloxicam in 10g saline solution, alkalized with 0.1g 20% NaOH. Depending on the dilutions absorbence at the wavelength 362nm, the calibration curve was plotted (slope = 0.182418223).

The maximum speed of diffusion rate of Meloxicam can be observed for the formula that contains Triethanolamine as increasing solubility agent. The maximum speed (0.018mg/min) was reached at about 10 minutes after application.

For the formula containing Tween 80, the peak is observed at 10 minutes after application, but is much lower compared with that of G2P2 formula.

The highest percentage of the diffused Meloxicam is met for G2P2 formula (90%), a formulation which have the highest values of the speed of diffusion. For the remaining formulas, the procentage of the diffused Meloxicam was of 40% as average.

The percentage of Meloxicam broadcasted at pH 4.5 was lower compared to that of acidic pH 4.5. The maximum procentage of total diffused Meloxicam was for the G2P2 formula.

### III.4.4. DISCUSSIONS

Like other NSAIDs, Meloxicam has been problematic due to its low solubility in the ointment base.

The diffusion rate (mg/ml) of Meloxicam through the artificial membrane increased a lot in all the experiments in the time interval 0-30 minutes, then gradually decreased. Quantities of Meloxicam could be detected even after a period of 24 hours after the ointment application on the artificial membrane.

The diffusion velocities obtained in Study no. II could be optimized by testing several diffusion enhancer agents. Of all the agents, triethanolamine had the greatest influence on the diffusion rate, which reached values of 0.018mg/min at 10 minutes after application. Peaks of the diffusion rates through artificial membrane using saline solution at pH 4.5 appear a little later, with a latency of 10-15 minutes compared with those seen when using physiological pH 7.5 saline solution.

### III.4.5. CONCLUSIONS

1. In all cases, the diffusion rate (mg/ml) of Meloxicam through artificial membrane increased dramatically in the period 0-30 minutes, then gradually decreased, quantities of Meloxicam being detected even after a period of 24 hours after ointment application on artificial membrane.

2. The maximum speed of diffusion of Meloxicam is recorded for the formulation containing Triethanolamine as increasing solubility agent. The maximum speed (0.018mg/min) is reached about 10 minutes after application. Evolution of the hydrogel G2P2 diffusion rate could be explained by the conversion of the Meloxicam in its salt, an easily soluble compound that can diffuse more easily through the artificial membrane.
3. By adding PEG 400 to the formula, the Meloxicam solubilised and led to the formation of a solution type ointment, but the development of the diffusion speed suggests a very high affinity between Meloxicam and PEG 400 or PEG 4000 for the formulations: G4 respectively U2.

4. The highest percentage of diffused Meloxicam diffused until the end of the experiment (24 hours after application of ointment), is met for the G2P2 formula (90%), a formulation which have the highest values observed in the speed of diffusion. For the remaining formulas, Meloxicam had an average in the procentage of diffusion of 40%.

**III.5. STUDY NO. III - THE PHARMACOKINETIC PROFILE OF IN VITRO RELEASE OF MELOXICAM THROUGH MOUSE SKIN FROM HYDROPHILIC SEMISOLID FORMULATIONS. INFLUENCE OF THE ABSORPTION PROMOTERS AND OF THE pH**

**III.5.1. AIMS**

This experiment aims to determine the *in vitro* pharmacokinetic profile of Meloxicam as a class representative of the NSAIDs using a natural membrane (skin of mouse) and the protocol from the previous study.

From the wide range of preparations obtained, in this study, the formulas with the best release profiles of the active substance were selected to be tested.

**III.5.2. MATERIAL AND METHOD**

The materials and utensils used had the same origin as those used in the previous studies. Freshly harvested from adult mice portions of skin were used as a natural biological membrane.

**III.5.3. RESULTS**

In the conducted experiment, it is noted that the G2P2 formula has the lowest percentage of Meloxicam remained in the skin (20%). Even with an 4.5 acidic pH, although values are slightly higher, the same tendency is kept - the G2P2 formula has the lowest percentage (25%) of undiffused Meloxicam.

The graphs represent the evolution of Meloxicam diffusion rate for the four formulations with different compositions in two different working conditions: pH 4.5 and pH 7.4.

**III.5.4. DISCUSSIONS**

The *in vitro* diffusion through animal skin of Meloxicam was more difficult than through artificial membrane. Meloxicam diffusion speed reaches higher values when working at physiological pH, compared with acidic pH, where values obtained were of approximately 1.1 g/min. The behavior at the two different pH was similar and the weight losses were roughly equal. Meloxicam losses observed in some cases may be due to the high affinity of Meloxicam for solubilization agent polyethylene glycol 4000.
III.5.5. CONCLUSIONS

1. The formula that have achieved the highest values of Meloxicam diffusion rate (mg / min) was G2P2 (carbopol gel with triethanolamine), a fact explained by increasing solubility of Meloxicam. Evolution of the hydrogel G2P2 diffusion rate could be explained by the conversion the Meloxicam into its salt with triethanolamine, a compound more soluble and thus that can easily run through natural membrane.

2. For the diffusion through the skin of mice, two peaks of the diffusion rate can be noticed: at 5-10 minutes and at 3 hours after starting the experiment. These peaks appear in all four tested formulations, the maximum being attained for G2P2 formulation. They could be due to different diffusion mechanisms involved in the transport of the active substance.

3. Percentage of Meloxicam diffused by the skin of mice ranged between 30 and 70%, with better values when using neutral pH and triethanolamine as solubilizing agent (G2P2 formula). However, compared with the literature data, the in vitro diffusion rates were lower.

III.6. STUDY NO. IV - COMPARISON OF IN VITRO MELOXICAM DIFFUSION THROUGH ARTIFICIAL MEMBRANE AND ANIMAL SKIN

Using data obtained in previous studies performed on artificial membrane and on dorsal skin of mice, some conclusions on the pharmacokinetic profile of Meloxicam circulated through various types of natural or artificial membranes using in vitro tests can be drawn.

Pharmaceutical formulation with the best results has been proved to be G2P2 containing 0.3% Meloxicam, 3% Triethanolamine, 1% Carbopol 990, 12% glycerol, 6.5% of 10% NaOH solution and 1% Fenosept solution (g/g).

Comparing the diffusion through the two types of membrane, the much higher values for the diffusion through the membrane artificial compared to animal what was expected, since the characteristics (thickness, composition, types of diffusion involved) of the two types of membrane are different. The diffusion speed through animal skin has two peaks at 5-10 min and at 3 hours after application, unlike artificial membrane where it reaches up to 15 minutes, then starts to fall until 24 hours. It should be borne in mind that the artificial membrane can have a thickness of up to 10 times lower than skin harvested from mice.

There were not significant differences in the use of pH 4.5 compared to the physiological pH.

III.7. STUDY NO. V IN VIVO PHARMACOKINETIC EVALUATION OF TOPICAL NSAID PREPARATIONS USING ANIMAL EXPERIMENTS

III.7.1. AIMS

In this study, I wanted to determine the plasma concentrations of some of NSAIDs after percutaneous administration of a single dose using laboratory animals and monitor their plasma concentrations within 24 hours. Qualitative and quantitative determinations were performed by liquid chromatography.

The study has also proposed to determine:
- If after the cutaneous administration the NSAIDs are well absorbed and reach plasma levels similar to the plasma levels described in the literature as therapeutic;

- If slow-release preparations administered in a single dose on skin have a good efficiency and tolerability.

**III.7.2. MATERIAL AND METHOD**

Adult male rabbits of almost identical weights (3 kg): Diclofenac - Diclac Hexal 5% gel, lot 8C7627, indomethacin - 4% indomethacin cream MARK Pharmaceuticals, lot 008, sterile dressings, syringes, disposable needles to collect samples, wool, sterile dressings, sterile containers to collect samples of blood, 90mg/10ml saline solution ZENTIVA, electric clipper, monopotassium phosphate Merck, acetonitrile Merck, chloroform, Merck, naproxen, electronic balance, centrifuge, Finnigan - SURVEYOR BDS Column - Hypersil C18, 250X4, 5 μm particle size liquid chromatograph.

The method used adult rabbits which were applied in the dorsal shaved skin a certain amount of industry produced and distributed in the pharmaceutical market in Romania semisolid pharmaceutical. Chromatographic samples were analyzed using an isocratic elution method.

**III.7.3. RESULTS**

After chromatographic analysis, the results were plotted.

**III.7.4. DISCUSSIONS**

Analgesic and anti-inflammatory action and toxicity are correlated with plasma drug concentration and are closely related to inhibition of cyclooxygenase. In this study, I determined the plasma concentration, an important pharmacokinetic parameter, after topical application in rabbits of the two anti-inflammatory drugs: Indomethacin and Diclofenac. The Diclofenac and Indomethacin had a similar progressive increase in serum levels up to 12 hours, but maintained a progressive decrease 24 hours after application for both preparations used.

Both substances have achieved similar plasma concentrations.

Since the testing was done using rabbits of the same sex, weighing approximately equal, the administration was carried out using the same route, skin, plasma levels of the substances should not be influenced by changes in absorption and first pass.

Animals showed no skin abrasions that could affect the absorption.

At the end of the study, the general condition of animals was good, no irritation, hematoma or infection signs were seen at the injection site. During the experiment, rabbits showed no adverse reactions (skin rashes, digestive disorders, anaphylactoid reactions).

**III.7.5. CONCLUSIONS**

1. The results of this study support the idea that the plasma concentrations of some NSAIDs (diclofenac, indomethacin) after topical administration on rabbit is close to levels obtained after oral dosing, but with a little delay.

2. Because of its mechanism of action, even after topical applying, the digestive contraindications of NSAIDs should be maintained and measures to protect the stomach must be taken, because plasma concentrations are high enough to achieve therapeutic systemic effects.
IV. FINAL CONCLUSIONS

1. Increased solubility of Meloxicam (a substance with poor solubility in lipophilic and hydrophilic solvents) was observed by adding 0.1% Tween 80, 30% Triethanolamine or 3% polyethyleneglycol 400, the only ingredients from all that were tested in this thesis that led to the solubilization of Meloxicam and to obtaining transparent solution type ointments;

2. In order to determine the in vitro kinetic profile of Meloxicam through artificial membrane, preparations with optimum characteristics were chosen, namely: gels: G1, G2P2, G4 and ointments: U1 and U2. The maximum speed of diffusion of Meloxicam was recorded for the formula containing the solubility enhancer agent Triethanolamine. The evolution of the hydrogel G2P2 diffusion rate could be explained by the conversion of Meloxicam into its salt with triethanolamine.

3. Two peaks were observed for the diffusion speed: at 5 to 10 minutes and at 3 hours after the beginning of the experiment of in vitro diffusion through the mice skin. They could be due to the different diffusion mechanisms involved in the transport of active substance.

4. In the study no V, the in vivo plasma concentrations after topical application of ointments with diclofenac and indomethacin in rabbits was determined by high pressure liquid chromatography method. It was observed that plasma concentrations is close to levels obtained after oral dosing, but a little delayed.

5. Because of its mechanism of action, even after topical application, the digestive contraindications of NSAIDs should be maintained and measures to protect the stomach must be taken, because plasma concentrations are high enough to achieve therapeutic systemic effects.
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