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In vitro and in vivo percutaneous absorption of topical dosage forms: case studies

I. Csóka[a,e], E. Csányi[a], G. Zapantis[a], E. Nagy[b], A. Fehér-Kiss[c], G. Horváth[b,c], G. Blazsó[d], I. Erős[a,*]

[a] *Department of Pharmaceutical Technology, University of Szeged, Szeged, Hungary*
[b] *Department of Physiotherapy, University of Szeged, Szeged, Hungary*
[c] *Department of Physiology, University of Szeged, Szeged, Hungary*
[d] *Department of Pharmacodynamics and Biopharmacy, University of Szeged, Szeged, Hungary*
[e] *Department of Drug Regulatory Affairs, University of Szeged, Szeged, Hungary*

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Abstract

This article evaluated the influence of vehicle compositions on topical drug availability. In vitro drug release and in vivo experiments were performed in case of the hydrophilic ketamine hydrochloride and the lipophilic piroxicam. Ketamine hydrochloride is a NMDA receptor antagonist that has been useful for anesthesia and analgesia. The study of transdermal ketamine delivery is a novelty, because nobody has investigated the hypnotic effects of ketamine after this administration route. In vitro measurements gave a good basis for screening among the developed products. The physiological changes after ketamine administration showed, that there were significant differences among the parameters tested (breathing rate, duration of sleep) from the developed products (hydrogel, lyotropic liquid crystal and o/w cream) compared to the reference product (Carbopol gel). The in vivo feedback for piroxicam was the measurement of the anti-inflammatory activity by edema inhibition percentage. Significant differences were measured in case of the developed systems compared to the reference.

Keywords: Drug release; Percutaneous absorption; Hydrogels; Lyotropic liquid crystals; Transdermal ketamine; Piroxicam

1. Introduction

A continuous interest toward the dermal and transdermal products can be seen, offering several advantages (Foldvari, 2000; Asbill and Michniak, 2000; Barry, 2001a). Data from USA shows that out of 129 drug delivery candidates, 51 transdermal or dermal sys-

* Corresponding author. Tel.: +36 62 545 570; fax: +36 62 545 571.
E-mail address: eros@pharma.szote.u-szeged.hu (I. Erős).

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tem products are listed; and 30% of 77 candidate products in preclinical development represent such drug delivery (Barry, 2002). Novel approaches, devices have been published in recent years (Asbill and Michniak, 2000; Barry, 2001b); parallel with the development of in vitro and in vivo test methodologies (Neubert and Wohlrab, 1990; Segers and Zatz, 1998; Hanson, 1989; Kierstan et al., 2001; Wissing and Müller, 2002) and mathematical description of the processes involved (Yogeshvar and Guy, 2001; Bunge, 1998). Studying the multistep process of drug delivery through the skin involves many different methodologies both in vitro and in vivo (Leveque et al., 2004).

Bioavailability study of a topical formulation begins with the in vitro investigation of the drug release from the compositions under evaluation (Abdou, 1989). In vitro release testing of the active ingredient has drawn much attention as a result of the edition of the SUPAC-SS (FDA Guideline, 1997), however as it was pointed out by FIP/AAPS (Siewert and Dressman, 2003), there was no standard test protocol that can be applied to all formulations. Data resulting from these investigations can be used as quality indicators, and also for the screening of the compositions prior to in vivo animal testing. Evaluation must be taken with care to avoid excessive extrapolation, as there are many anatomical and physiological factors, which are not represented under these circumstances, in spite of the very fast development of this field (Thakker and Chern, 2003).

The selection of the drugs and dosage forms were based on the following demands. The need for transdermal ketamine delivery rose up from the clinicians' side. It is well known, that induction of anaesthesia by inhalation or injection is a stormy procedure in children. In addition, painful and frightening experiences may cause long-term psychologic complications and make subsequent contacts with health professionals more difficult. As a result, a variety of premedications administered via various routes have been introduced, e.g. rectal administration of ketamine (Tanaka et al., 2000). It has been shown that rectally administered ketamine alone produced dose-dependent sedative effects in children. Only a few studies reported about the antinociceptive potential of the transdermal ketamine (Crowley et al., 1998; Quan et al., 2003; Azevedo et al., 2000), but nobody has investigated the hypnotic effects of this drug after local administration. Quan et al., (2003) have shown that topical ketamine reduced pain in patients with no systemic side effects, indicating negligible or no generalized absorption. Azevedo et al., (2000) have found that a controlled transdermal delivery of ketamine prolonged the time to first rescue analgesic medication without adverse effects after minor gynecological surgery.

Topical administration of non-steroidal anti-inflammatory drugs (Takahashi et al., 2002), including piroxicam is important from many points of view and has been investigated by several researchers (Shin et al., 2000; Doliwa et al., 2001; Takahashi et al., 2002). Our interest turned towards this active agent to fill the need for a well-penetrating, easy spreadable, washable, and if possible transparent product to extend the present product range. The dosage forms used belong to a relatively newer group of semisolid systems offering a very good patient-complience and low irritation potential (Brinon et al., 1999; Peppas et al., 2000) to sensitive skins. Compositions presented here were developed by our research group (Makai et al., 2003; Erős et al., 2003). The effects of surfactant presence in the formulations on the biological systems are well known (Attwood and Florence, 1982).

2. Materials and methods

2.1. Materials

The following materials were used as vehicle components. The lipophilic phases, liquid petrolatum and isopropyl myristate were purchased from Hungaropharma Co., Budapest. The amphiphilics used were as follow: cetostearyl alcohol (Hungaropharma Co., Budapest), Polysorbate 80, Brij 96V (ICI Hungary Ltd., Budapest,). The polymers—Carbopol 940 and 971P were donated by S&D Chemicals Ltd., Budapest. All the other additives, triethanolamine, carbamide, sodium hydroxide and glycerol were obtained from Hungaropharma Co., Budapest. All components used were of Ph. Eur. 4th grade.

2.2. Preparation of the formulations

Carbopol 940 was added to the water phase and set for 10 min; this mixture was neutralized by means of sodium hydroxide in order to get the reference gel. In case of the hydrogel, Carbopol 971P was used as poly-

mer, and it was added first to distilled water. The carbamide was dissolved in the remaining water phase, followed by adding triethanolamine for neutralization. The lyotropic liquid crystal samples were produced by heating the mixture of the liquid petrolatum, the glycerol and of Brij 96V to 80 °C. Distilled water was heated up to the same temperature and was added during constant stirring at 500 r/s (Ikamag RET-G magnetic stirrer). Stirring was continued until the mixture cooled down to room temperature. The preparation of the o/w cream was performed as follows: Polysorbate 80, cetostearyl alcohol and isopropyl myristate were melted together (80 °C) and mixed. The aqueous phase containing carbamide was then heated up to similar temperature. Finally the phases were mixed and homogenized. Ketamine was dissolved in all of the cases, while piroxicam was suspended in the vehicles.

2.3. In vitro release testing methods

Franz glass diffusion cell system (Hanson Research Co., USA) containing six cells, and equipped with autosampler (Hanson Microette Autosampling System) was used. The area for diffusion was 1767 cm^2, and the receptor chamber volume was 7 ml. Cellulose acetate membranes (Porafil, Machenerey-Nagel, Germany) with an average pore size of 0.45 μm were used. Pretreatment of the membrane by soaking in the receiving medium and/or isopropyl myristate (IPM) were performed. Membrane filters were mounted to Franz diffusion cells. The experiments were run at 32 ± 0.5 °C. The receptor medium was phosphate buffer (pH 5.4). A receiving medium that is similar to the physiological condition of the skin was selected, including the practical consideration to choose a receiving medium that allows sufficient amounts of active ingredient released within a reasonable time period to ensure accurate analysis. 0.40 g samples of different compositions were placed evenly on the surface of the membrane, and 800 μl samples were taken after 0.5, 1, 2, 3, 4, 5, and 6 h and replaced with fresh receiving medium. The absorbance was measured by UV-spectrophotometer (Unicam Helios α UV–vis Spectrophotometer, England) at 269 nm in case of ketamine, and at 359 nm when piroxicam was incorporated, based on prior calibration curves. The blank vehicles without active agents served as references in the analytical measurements.

The cumulative release of ketamine and piroxicam (1% w/w) over a 6 h time period were plotted according to the diffusion model of Higuchi, (1962). The average of four experiments is summarized in the figures.

2.4. In vivo percutaneous absorption studies: ketamine

After institutional approval had been obtained from our animal care committee, male Wistar rats weighing 240 ± 4.8 g were studied. The animals were kept on a 12 h light/12 dark cycle with food and water ad libitum. All experiments were carried out in the same period of the day (1–4 p.m.) to exlude diurnal variations in pharmacologic effects. Each rat was tested only once. One day prior to the application of the cream, the back of each rat was carefully shaven and the skins are cleaned by wiping with water containing cotton under seduxen–xylazin anesthesia. On the day of the experiment, the animals were anesthesized with the intraperitoneal injection of Seduxen® and xylazine (1 mg/kg and 10 mg/kg intraperitoneally, respectively). Each cream (1.0 g) was applied onto the dorsal skin of rat (about 15 cm^2/kg) 5 min after the injection, and covered with a protective overlay, an elastic adhesive bandage. The nonwoven PE cloth was fixed with Coban® adherent wrap. Control animals were exposed to the placebo cream (vehicle without active agent) in the same amount. The cream and the overlay were removed after the termination of the experiment.

Loss of the righting reflex was used to determine the presence of anesthesia, and its length in minutes was referred to as the duration of hypnosis. This technique is widely used for the assessment of anesthesia in rodents, and good agreement has been observed between the results with general anesthetics presented in various papers (Miller et al., 1972; Horvath et al., 1992). Hypnosis was regarded as the state in which an animal could be placed on its back without righting itself. During the anesthesia the breathing was also determined in every 5 min (breathing frequency).

The rats were treated randomly according to one of the following protocols: controls received vehicle (n = 10); experimental animals were exposed to different vehicles (hydrogel, liquid crystal, o/w cream) (n = 4–7) containing 1% (w/w) ketamine. Data are presented as means ± S.E.M. The statistical analysis of difference between different treatments was performed

Table 1
Composition of the investigated preparations (%, w/w)

Component	Reference gel	Hydrogel	Liquid crystal	o/w cream
Liquid petrolatum	–	–	10	–
Isopropyl myristate	–	–	–	20
Triethanolamine	–	2	–	–
Tween 80	–	–	–	8
Brij 96V	–	–	42.5	–
Glycerine	–	–	17.5	–
Carbopol 940	0.6	–	–	–
Carbopol 971 P	–	1	–	–
Carbamide	–	5	–	10
Sodium hydroxide	0.18	–	–	–
Cetylstearyl alcohol	–	–	–	12
Ketamine hydrochloride	1	1	1	1
Distilled water	Ad 100	Ad 100	Ad 100	Ad 100

with the Student *t*-test. A probability level less than 0.05 was considered as significance.

2.5. In vivo percutaneous absorption studies: piroxicam

Male Sprague–Dawley rats weighing 200–220 g were depilated on the back (approx. 20 cm^2) by Veet® (Reckitt Benckiser, France) depilatory cream. The animals were randomly assigned seven groups, with six rats in each. Three hours after the depilatory procedure, 300 mg preparation, with or without piroxicam was applied onto a 15 cm^2 surface of the depilated skin back of each rat. Three groups served as vehicle controls, and one was not treated at all. The three remaining groups were treated with different vehicles containing piroxicam in concentration of 1% w/w. Local inflammatory response was elicited by 0.1 ml subplantar injection of carrageenin (Viscarin, Marine Colloids Inc., Springfield, USA) solution given into the right hind paw one hour after the treatment. The contralateral foot was injected with isotonic saline. The concentration of carrageenin solution was 0.5%. The rats were kept at controlled ambient temperature (22.0 ± 1.0 °C). Rat chow and tap water were supplied ad libitum. The experiments were conducted in accordance with the institutional guidelines of the University of Szeged, Hungary.

Edema formation was measured by plethysmometer (Hugo Sachs Elektronik Gmbh, Germany) 5 h after the subplantar injection of carrageenin. The volume difference between the carrageenin- and saline-injected paws was used for the evaluation of the inflammatory response. Results are presented as means ± S.E.M. Statistics were performed with one-way ANOVA followed by Dunett's test. Statistical significance was accepted at a probability level of $p < 0.05$.

3. Results and discussion

The composition of the investigated formulations is shown in Table 1.

3.1. In vitro release of ketamine hydrochloride

The release process from different vehicles was measured first through a synthetic membrane, which was soaked in the acceptor phase. Fig. 1 shows the cumulative released ketamine amount plotted against $\sqrt{t}$

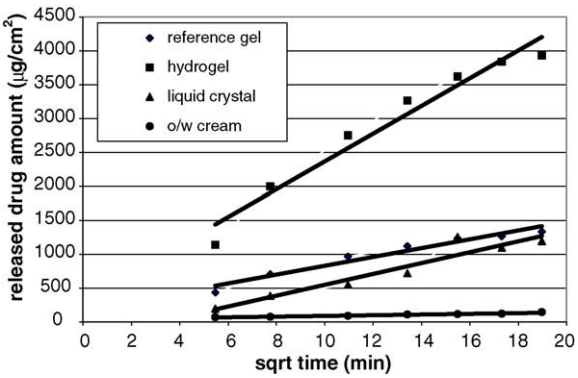

Fig. 1. Cumulative released ketamine through synthetic membrane soaked in buffer solution.

Table 2
Comparison of the diffusion rates, correlation coefficients and the cumulative released drug amounts after 6 h measured in case of synthetic membrane soaked in buffer solution

Compositions	Diffusion rate (slope of the lines)	Correlation coefficients	Released drug amount during 6 h (μg/cm^2)
Reference gel	65.07	0.9537	1334.1
Hydrogel	204.93	0.9589	3927.9
Liquid crystal	80.286	0.91	1197.0
o/w cream	5.0801	0.96	142.6

for the determination of the release rate from the lines. Table 2 summarizes the slopes representing the diffusion rates and also the released drug amounts after 6 h, are comparised. A hydrogel > reference gel > liquid crystal > o/w cream order was set based on the fluxes measured from the developed products. Differences between these rates are mainly due to the differences in the rheological parameters (unpublished results, not presented here) of the systems as both the dispersed state of the drug (dissolved), and its concentration were constant in this study.

When impregnating the membrane with IPM, the following changes were detected (Fig. 2.)

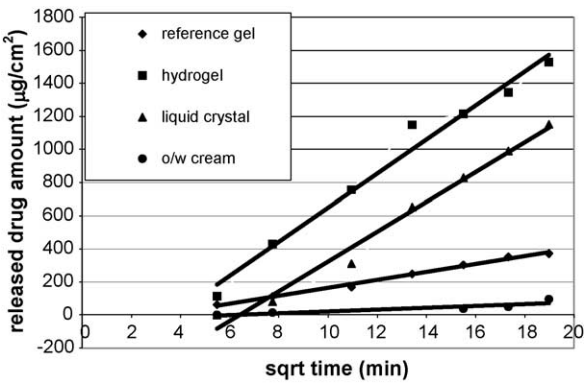

Fig. 2. Cumulative released ketamine through synthetic membrane soaked in IPM.

The parameters of the data presented here are listed in Table 3.

Reference gel and hydrogel fluxes decreased significantly when the membrane was soaked in IPM, while liquid crystal system with high surfactant content did not show a significant change. The presence of penetration enhancers in the developed products is well manifested, which is promising and shows ability to alter skin permeability properties as well. While drug release through synthetic membrane was mainly influenced by the rheological properties of the vehicles, diffusion ability through IPM membrane is the consequence of enhancer present in the formulations.

3.2. In vivo percutaneous testing results of ketamine

Products containing 1% ketamine, selected on the basis of the in vitro experiments were evaluated by different physiological tests. Fig. 3 shows the effect of ketamine containing products on breathing frequency, on the asleeping time and on the duration of sleep. The time needed for the appearance of the first urine was also detected.

There were no significant differences between the groups in respect of the onset of hypnosis, however the duration of the hypnosis significantly increased in the ketamine developed products compared to the reference product. The breathing frequency significantly decreased in the ketamine developed product group

Table 3
Comparison of the diffusion rates, correlation coefficients and the cumulative released drug amounts after 6 h measured in case of synthetic membrane soaked in IPM

Compositions	Diffusion coefficient	Correlation coefficients	Released drug amount after 6 h (μg/cm^2)
Reference gel	23.995	0.9024	372.1
Hydrogel	102.9	0.9801	1528.72
Liquid crystal	89.824	0.984	1150.212
o/w creams	5.5557	0.8169	95.728

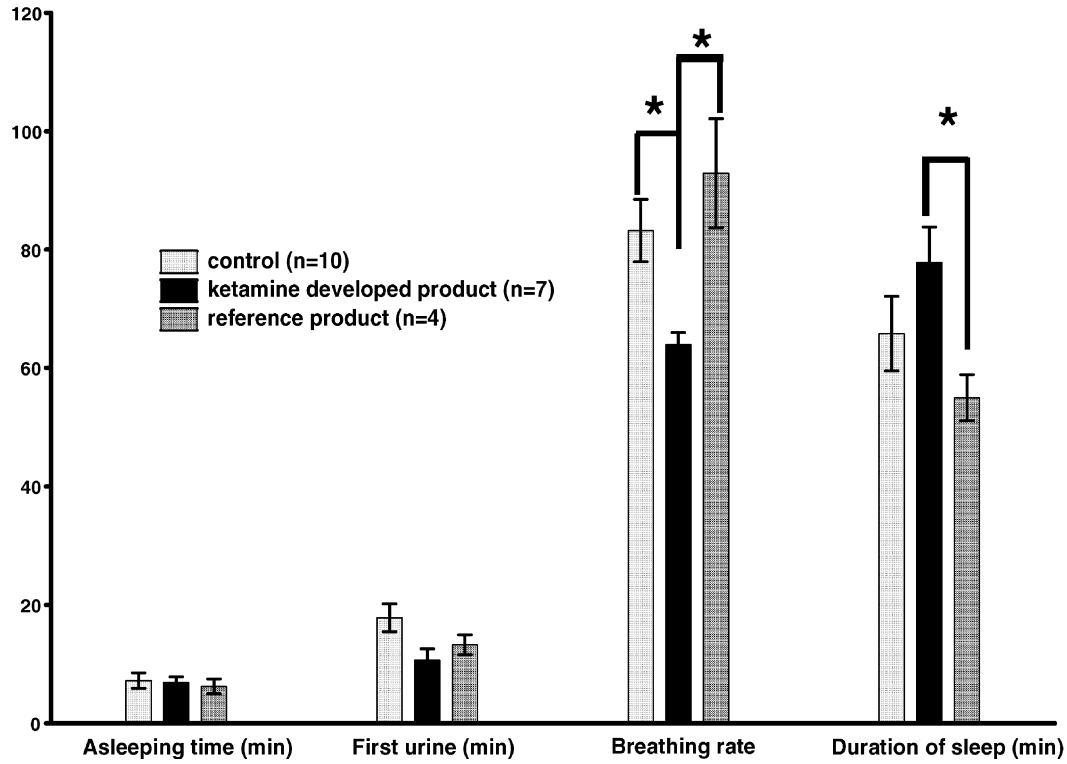

Fig. 3. The in vivo effect of ketamine on the different physiological parameters in anaesthetized rats. * = significant difference.

compared to the other two groups (reference gel and control group receiving vehicles without active ingredient). We have found that the in vivo effect of developed products (hydrogel, liquid crystal and o/w cream) did not differ significantly; therefore these data have been merged into one group.

Our results have shown that the developed products (hydrogel, liquid crystal and o/w cream) containing ketamine in 1%, had a significant potency in in vivo circumstance, while the reference gel did have neither any potentiating effect on the duration of the hypnosis nor significantly influence in the breathing frequency.

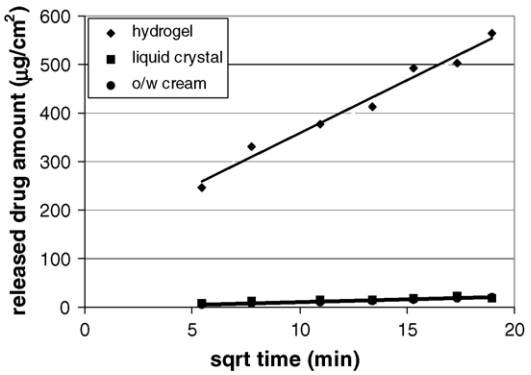

Fig. 4. Cumulative released piroxicam through synthetic membrane soaked in buffer solution.

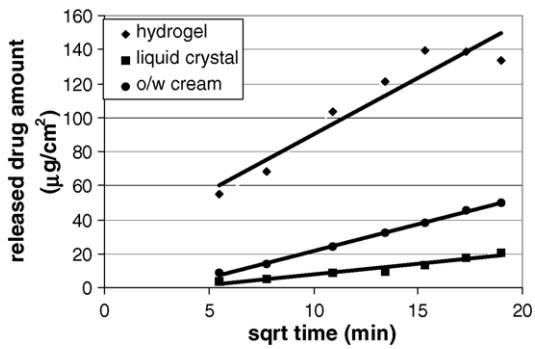

Fig. 5. Cumulative released piroxicam through synthetic membrane soaked in IPM.

Table 4
Comparison of the diffusion rates, correlation coefficients and the cumulative released drug amounts after 6 h measured in case of synthetic membrane soaked in buffer solution

Compositions	Diffusion rate (slope of the lines)	Correlation coefficients	Released drug amount during 6 h (μg/cm^2)
Hydrogel	21.965	0.9761	564.39
Liquid crystal	0.8518	0.8545	18.97
o/w cream	1.0934	0.9899	20.52

Table 5
Comparison of the diffusion rates, correlation coefficients and the cumulative released drug amounts after 6 h measured in case of synthetic membrane soaked in IPM

Compositions	Diffusion rate (slope of the lines)	Correlation coefficients	Released drug amount during 6 h (μg/cm^2)
Hydrogel	6.6433	0.9106	133.68
Liquid crystal	3.1245	0.9976	20.31
o/w cream	1.2263	0.9534	49.98

3.3. In vitro release fluxes of piroxicam

Piroxicam release measured through synthetic membrane soaked in buffer solution gave the following results (Fig. 4). Table 4 illustrates the parameters of the drug release measurements.

Significantly higher amounts of piroxicam released during the investigation period, as seen from the table. Similar amounts of drug were measured in the acceptor phase after 6 h of the experiments in case of the liquid crystal system and the o/w cream.

The piroxicam flux from the hydrogel decreased (Fig. 5), when measured through IPM soaked membrane, while the released drug amount remained constant from the lyotropic liquid crystal system, compared to the unsoaked membrane. Double amount of drug was detected from o/w cream in comparison with the liquid crystal system, as seen in Table 5.

3.4. In vivo anti-inflammatory activity of piroxicam

The carrageenan-induced oedama test was used to test the in vivo efficiency of the developed formulations. Data are presented in the Fig. 6. The liquid crystal and hydrogel without piroxicam (placebo) had not any statistically significant anti-inflammatory effect. The placebo o/w cream showed a weak inhibition on swelling when compared to the "without treated" control group ($p < 0.01$). The groups treated with piroxicam 1% w/w in liquid crystal or hydrogel could elicit a very effective anti-inflammatory effect ($p < 0.001$) but piroxicam 1% (w/w) in o/w cream showed a mod-

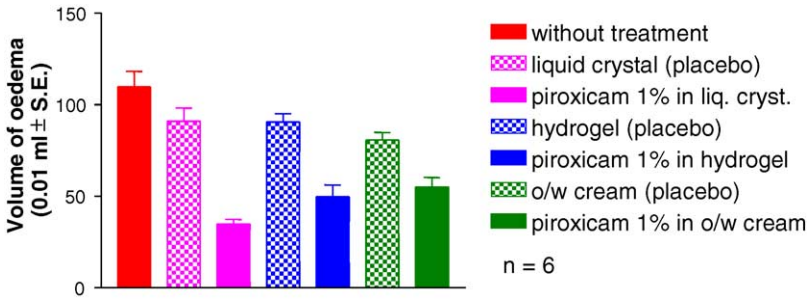

Fig. 6. Antiinflammatory effect of different preparations on carrageenan-induced oedema in rat.

erate effect (0.01) when compared to the placebo groups.

4. Conclusions

The transport of ketamine hydrochloride and piroxicam through skin was examined from different vehicles. In vitro drug release studies were made by means of Franz diffusion cell system using synthetic membrane soaked in buffer solution or in isopropyl myristate. The products for in vivo testing were selected based on these results. In vitro skin permeation studies are presently under evaluation. In vivo studies with ketamine show that while there was no significant change in the onset of action, significant changes were detected in the duration of hypnosis and in the decrease of the breathing rate.

In case of piroxicam, the liquid crystal system and the hydrogel showed to be efficient, while o/w cream exerts only a moderate oedema inhibition. Detection of plasma levels in the course of time is also ongoing to follow the whole process.

No correlation was found between the orders of preparations set up after the in vitro results comparing to that of in vivo efficiency studies.

Histological examinations with light microscopy for vehicle effect assessment are going to be performed to complete these studies.

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