

## ABSTRACT

Non steroidal anti - inflammatory drugs (NSAIDs) are substances with the quality to reduce pain and the signs and symptoms of an inflammation, yet, without removing the causes that produced it. In veterinary medical practice their place is mainly bounded with the incidence of musculo-osteo-articular disorders, diseases with a high incidence in dogs, these substances being also used in post operative allergies.

The PhD thesis entitled: *The pharmacodynamic study regarding the therapeutical action of the non steroidal anti inflammatory drugs (NSAIDs) – cyclodextrins complex in animals*, contains a number of 261 pages and is structured according to actual legal regulations in two main parts: first part – *Present stage of knowledge*, with a number of 51 pages, representing 21,3 % and second part – *Personal researches*, with a number of 188 pages, representing 78,7 % from the volume of thesis.

**First part**, structured by 3 chapters, contains the succinct information from scientifically papers related to the thesis subject that were ulterior used in part two as reference data for interpreting and discussions of the results. In the *first chapter*, entitled *Inflammation and inflammatory response*, are suggestively presented dates from the speciality literature that are relevantly for the physiopathology of the inflammatory process, a etiology and pathogenic phenomenon of the inflammation, local modification that appear, chemical mediators and inflammation classification from etiologic and evolutionary point of view. In the pathogenic process of the inflammation we may distinguish: a trigger mechanism, a chemical mediation stage and a terminal stage, or prostaglandin stage. The pathologic process that develops in healthy tissues, at the breeding ground of aggression agent (inflammatory focus), characterized by alterative, vascular-exudative and proliferative phenomena, is followed by a general reaction of the organism, unspecific, with defence - adaptation character. The purpose of these modifications, in which takes part the organism as a whole, is to neutralise and remove the pathogen agent, followed by the repairment of the injured tissues.

*Second chapter*, entitled *The pharmacology of the non steroidal anti-inflammatory drugs*, presents bibliographic dates related to therapeutical action of this class of medicamentous

substances in inflammatory process and side effects that they induce inside the organism. Non steroidal anti-inflammatory drugs (NSAIDs) are substances included in analgesic – antipyretic – anti inflammatory group and they are used for the therapy of different inflammatory disorders with decreased till mild intensity, but also in some severe pain disorders such as renal, biliary or rectal colic. The mechanism by which these substances work as anti inflammatory drugs is mainly due to inhibition of the cyclooxygenases (COX 1, COX 2, COX 3), enzymes that play a role in prostaglandins synthesis.

Wide use of NSAIDs in veterinary practice is relatively limited by side effects (gastrointestinal, renal, cardiac, hepatic etc.). This relative limitation of their use in veterinary medicine in comparison with human medicine has its explanation in the pharmacokinetic parameter differences in different animal species, therefore creating the possibility to limit the therapeutical effect or to have an excessive toxicity.

Nowadays, there are many trials for discovering less toxically NSAIDs, such as high selective inhibitors of COX-2, and as caution measures, there are recommended association of *ranitidine* or *cimetidine* in prophylactic doses, *misoprostol* administration, an analog for PGE1 (both prophylactic and curative), yet, all these including higher cost of the therapy. More recently and with multiple advantages, in pharmaceutical industry are used ***the cyclodextrins*** to form inclusion complex compounds with different medicamentous substances.

In ***chapter III*** from the first part, entitled *Importance of the inclusion compounds use*, contain bibliographic dates that describe the physical-chemical proprieties of the cyclodextrins, their utility and applicability in pharmaceutical industry, as well as the importance of making and using polymer – drug conjugated systems in the context of present drug scientifically evolution. Medical, biologic and biomedical sciences registered amazing progresses in the last decade, opening new understanding pathways for both normal and pathologic processes and creating more specific and efficient new medicamentous systems.

**Second part**, describes the personal researches, is structured in 8 chapters and contains the aim and orientation of the researches, study materials and used methods, obtained results and their interpretation, ending with general conclusions.

***Chapter IV – Aim and the objectives of the research*** – underlines the motivation of this research, describing the objectives of the thesis. The research hypothesis started from the premise that in inflammatory disorder with osteoarticular origin in pets (dogs), often with an

invalidated evolution, are frequently used non steroidal anti inflammatory drugs, that beside their benefits have the risk of digestive reactions. The main aim of the PhD thesis was the improvement of the pharmaceutical presentations belonging to non steroidal anti inflammatory class, by complexation with  $\beta$ -cyclodextrin, in order to obtain new products with different features such as increased solubility and bioavailability and elimination of toxic effects. The main objectives followed in the research process, are represented by:

- comparative evaluation of the efficacy and tolerability of the non steroidal anti inflammatory drugs in dog;
- biocompatibility assessment of  $\beta$ -cyclodextrin;
- obtaining the non steroidal anti inflammatory drugs with  $\beta$  – cyclodextrin inclusion compounds;
- making the *in vivo* experimental models in order to evaluate the anti inflammatory effect of the substances complex with  $\beta$  cyclodextrin;
- efficacy assessment of carprofen and ketoprofen complex with beta cyclodextrin in comparison with their activity in uncomplex form, on experimental inflammation models in laboratory animals;
- biocompatibility comparative assessment of the carprofen and ketoprofen complex substances and uncomplex drugs by oral administration in rats;
- clinical assessment of the efficacy of substance complex with beta-cyclodextrin administered in dogs.

In **chapter V** – are comparatively described the anti inflammatory effect and tolerability degree of 3 substances from non steroidal anti inflammatory drugs class – carprofen, meloxicam and ketoprofen, frequently used in veterinary practice in dogs that suffer different inflammation disorders with osteoarticular nature. The studies took place in three private veterinary clinics, on a number of 60 dogs, divided in 3 groups with 20 patients each group and that needed non steroidal anti inflammatory drugs therapy.

The obtained results proved the efficacy of each tested anti inflammatory substance, by pain remission, decrease in the intensity of the inflammatory process and recovery of the locomotors functions, but the highest percentage obtained to improve the phenomena was observed after meloxicam therapy – 80%, followed by ketoprofen – 60% and carprofen – 50%. The tolerability degree, observed during therapy period, showed that all studied NSAIDs –

carprofen, meloxicam and ketoprofen, were marked by digestive side effects, like vomiting and diarrhea. The tolerability degree was labeled as being good in 32% and weak in 68% of patients, fact that proves a high incidence of side effects under therapy with NSAIDs.

Evaluation modalities for  $\beta$ -cyclodextrin, a natural oligosaccharide obtained from starch synthesis, are described within **chapter VI**. There was tested both biocompatibility of  $\beta$ -cyclodextrin, as its acute toxicity by determining lethal dose 50 (LD50) in laboratory animals – Swiss line white mice. After administering  $\beta$ -cyclodextrin to mice there was observed neither mortality nor any changes of the general status or of the hematological and biochemical parameters. Biocompatibility tests underlined the fact that this substance was well tolerated by all mice, no matter of the administrated dose or concentration, or administration route. After acute toxicity assessment of  $\beta$ -cyclodextrin, the obtained result prove the fact that LD50 is equal to 12000 mg/kg body weight, and is labeled in 5<sup>th</sup> toxicity group after Hodge and Sternert, fact that indicated that the substance is practically not toxic for the organism.

The biocompatibility and acute toxicity testing for cyclodextrin had as goal making the inclusion complex compounds with substances from non steroidal anti inflammatory drugs class, in order to obtain products with an improved qualities, with a high efficacy and without the risk of side effects.

The technologies used to obtain inclusion complexes, described in **chapter VII**, which form a series of actions, have as final result the creation of a complex product from a medicamentous substance and a cyclodextrin, with highly improved pharmacologic proprieties. For making the non steroidal anti inflammatory drugs – cyclodextrin conjugated compounds was used the *liophilisation* technique, which allowed making the ketoprofen -  $\beta$  cyclodextrin and carprofen -  $\beta$  cyclodextrin inclusion compounds, using water as solvent. For the formed complexes, analyzed by quality test methods such as SEM, TG-DTG, DSC and solubility phase, significant differences were noted between the profiles of the obtained complexes and of the initial substance (ketoprofen, carprofen and  $\beta$ -cyclodextrin). The morphological structure changes, increase of thermal stability and concentration of active substances, together with solubility coefficient increase of ketoprofen and carprofen powder by significant improvement of dissolving process in water, are some of the advantages that came along with  $\beta$ -cyclodextrin complex.

In order to evaluate the anti inflammatory therapeutical efficacy of the new formed inclusion compounds: ketoprofen +  $\beta$  cyclodextrin and carprofen +  $\beta$  cyclodextrin, were created two *in vivo* inflammation experimental models, described in **chapter VIII**. The experiment were undertaken on male rats, in homogenous groups, to whom it was experimentally induced *plantar inflammation* with 10% kaolin watery suspension and *peritoneal inflammation* with 1% sodium thioglycolate watery solution. The subcutaneously injection of 10% kaolin suspension on plantar side of the posterior foot of rat produced an edema, evaluated by pletismometry, starting with approximately 1 hour from the induction of the inflammatory process and it had an intense growth over an 9 hours period. The maximal peak of the plantar inflammation was noticed after 9 hours and maintained at the same values till 12 hours, following a moderate subsequent decrease of edema, getting back to normal in approximately 4 days. The pletismometry technique allowed exact dynamic evaluation of plantar induced inflammatory process using 10% kaolin. The inflammatory process that appeared in experimental induced acute peritoneal inflammation was underlined by spectrophotometry and cellular concentration assessment from peritoneal liquid. The high spectrophotometry absorbance values, together with high cellular afflux from the peritoneal liquid samples, underlined an intense inflammatory process determined by intraperitoneal administration of 1% sodium thioglycolate solution.

The inflammation experimental models gave important results for anti-inflammatory efficacy evaluation of carprofen and ketoprofen complex with beta cyclodextrin, and of substances alone.

*Aspect regarding biocompatibility and efficacy of non steroidal anti-inflammatory – cyclodextrin conjugated compounds* are described in **chapter IX**. The studies were undertaken on 2 experimental series of rats – A and B series, which were separated in groups, each group being formed from 6 animals ( $n = 6$ ). The experiment was made on two different models of experimental induced inflammation in rats. The antiinflamamtory effect, for the plantar edema, of ketoprofen and carprofen complex with cyclodextrin was observed to be maximum at 5 – 6 hours after administration, determining an inhibition of the inflammation process of approximately 40% in comparison with the withness group where there was no reaction and 15% in the case of the groups treated with uncomplex substances.

The obtained results showed a significant difference of the anti-inflammatory efficacy for ketoprofen +  $\beta$  cyclodextrin and carprofen +  $\beta$  cyclodextrin complexes versus medicamentous

substances ketoprofen and carprofen alone, both for plantar and for peritoneal inflammation in all tested rats. One of the most important features of the inclusion complexes that was obvious in our studies, was the improvement of the anti inflammatory therapeutical effect, inducing a good tolerability, without the appearance of side effects at digestive, renal and hepatic level, which were induced by carprofen and ketoprofen alone.

*Chapter X-* entitled *Efficacy and tolerability assessment of ketoprofen -  $\beta$  cyclodextrin complex on inflammatory disorders in dog* reveals the advantages of using NSAIDs inclusion complexes with  $\beta$  cyclodextrin in the veterinary practice. The recovery rate of the dogs treated with the ketoprofen -  $\beta$  cyclodextrin inclusion complex was visibly better than of those which had been treated with the uncomplex substance. A decrease of the side effects in dogs treated with the inclusion complex was also noted, in comparison with the patients treated with the substance alone and had digestive side effects in 100 % of the cases. Good tolerability of the ketoprofene complex is probably also obtained because cyclodextrin has the ability to protect the drug molecule from gastric degradation and to release high levels of active substance in the intestins, which lead to increased absorbtion and bioavailability of the active substance in the organism, thus decreasing the toxic effects.

The final results of the experimental study have proven superior efficacy and safety in the use of cyclodextrin complex drugs for the treatment of inflammatory disorders in dog. The intensity of the anti-inflammatory effect produced by cyclodextrin - associated substances was significantly greater than that of the unassociated ones, because **cyclodextrin** is considered to have the role of increasing the bioavailabilty and solubilty of the conjugated substance.