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Design and pharmacokinetics of a new formulation of modified release of clindamycin orally for dogs.

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ABSTRACT

Clindamycin is an antibiotic by choose, because of its wade distribution into organs and tissues, it is used in the treatment of osteomyelitis, however the owners of dogs does not have the discipline necessary to complete the treatment, affecting therapeutic response.

The innovation of polymeric matrix made of hydroxypropylmethylcellulose (HPMC) + poloxamer 188 and/or made of HPMC + carbopol with the addition of clindamycin will result in modified release formulations, increasing therapeutic concentration time in serum, and decreasing dosing frequency.

Two formulations of 20 mg/Kg orally made of HPMC with poloxamer 188 and HPMC with carbopol were designed; they were compared with a control treatment of clindamycin without excipients. The sanguineous concentrations of clindamycin were determined by blood samples take it until complete sixty hours. The clindamycin concentration in the serum was determined by the diffusion method in Bennet agar, and the pharmacokinetic analysis was made with the software PKAnalyst. The results were analyzed by the ANOVA test, and the difference between the groups was analyzed by the Tukey test.

An innovative clindamycin design of controlled released was achieve, thanks to the addition of the excipients based on a polymeric matrix in their formulations.

Key words: canine, carbopol, hydroxypropylmethylcellulose, lincomycides, long-acting

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1. INTRODUCTION

Clindamycin is the antibiotic by choice to chronic treatments like osteomyelitis, with an orally administration twice a day with a treatment time of 15 to 21 days (1), however the medication can be an inconvenient, because the owners of the dogs does not have the discipline necessary to complete the treatment, affecting therapeutic response. Thus it requires to obtain concentrations above the minimum inhibitory concentration (MIC) of the pathogen to accomplish its therapeutic effect. Clindamycin has a wide distribution to organs and tissues and it has a low toxicity, but it has a very limited therapeutic effect in the organism (12 hours) (2).

In recent years an increasing number of new pharmaceutical ways of modified release have appeared, improving the pharmacokinetic profile and reducing the adverse effects of active ingredients, compared with conventional medicine of immediate release or conventional release (3).

The modified released is a system that releases a drug in a specific velocity and/or position according to the needs of an organism, during an specific period of time (4). With these factors in mind, it is possible to obtain release systems that act in a slow and continuous way during long periods of time (5). This research pretends to increase clindamycin serum permanence up to 48 hours, unlike the 12 hours duration of clindamycin with conventional release.

This work is going to formulate a polymeric matrix based on HPMC, it will be constituted by polymers with methyl groups and hydroxypropyl joined to an glucose anhydrous chain (6), HPMC is used in the preparation of oral and topical pharmaceutical forms, as binder in wet or dry granulation, and as a matrix of controlled release tablets (7). Poloxamer 188 will be used too, it is used in a variety of pharmaceutical formulations that can be administered orally, it is not considered toxic or irritating, this polymer allows developing both, formulations of instant release and controlled release (7). The carbopol is a synthetic polymer of that acts like a bioadhesive and like an agent of controlled release (7).

Methods:

The following materials were used, and they were generously donated by different manufacturing firms. Clindamycin hidrochloride (Halvet, México), Hidroxi-Propil-Metil-Celulosa (HPMC) (Vivapharm K -100) ® JRS Mexicana, Poloxámero 188 (Lutrol 127) ® BASF, Mexico City, and Carbopol ® 971 P NF polimero (Lubrizol, México).

The animals of this study was approved by "Subcomité Institucional de Investigación, Cuidado y Uso de Animales Experimentales" (SICUAE) of Universidad Nacional Autónoma de México in January 2014, in accordance with Norma Oficial Mexicana (8), was used 21 healthy dogs that stayed in the kennels of surgical education area of Facultad de Medicina Veterinaria y Zootecnia of Universidad Nacional Autónoma de México for two weeks; they were fed with dry commercial food and water ad libitum. Two weeks before to take samples, the rabies vaccine was administered (IMRAB T-3®, Merial), also polyvalent vaccine (RECOMBITEK®, Merial), and a dose of deworming medication.

Physico-chemical characteristics previous to formulation:

The physical and chemical characteristics of clindamycin hydrochloride powder were obtained by granulometry through electronic microscopy and by distribution of the particle size. As regards rheological properties, proofs of apparent density, compacted density, repose angle, flow rate, Carr's compressibility index, Hausner index, porosity percentage and dust moisture percentage, were made; the dust moisture percentage was measured through a thermobalance OHAUS MB 2000.

Formulation and Procedure:

For the preparation of controlled release of medicine, clindamycin hydrochloride, HPMC, poloxamer 188 and carbopol were mixed in two different formulations; both preparations have the same base of clindamycin blended with HPMC, the first formulation has poloxamer 188 unlike the second formulation that contains carbopol; these preparations will be named (CLI-H-P) and (CLI-H-C) respectively. A control group was considered, dosing with just a single formulation of clindamycin without excipients (CLI),

Treatment and Determination of Clindamycin activity:

The clindamycin it was mixed with the excipients previously mentioned, through the technique of granulation; after the entire formulated was mixed.

The animals were divided into three groups of formulations. The first formulation is called (CLI-H-P) and its had 20 mg/Kg of clindamicina, 40% of the final preparation of hydroxypropylmethylcellulose and 5.6 mg/Kg of poloxamer 188, the second formulation is called (CLI-H-C) and its had 20 mg/Kg of clindamicina, 40% of the final preparation of hydroxypropylmethylcellulose and 5% of carbopol in the final preparation, and the third formulation, was the control and is called (CLI) without excipients like the Table 1 shows, in all the treatments, the administration was orally.

The samples of 3 ml were obtained from jugular vein until complete 60 hours, later the samples were centrifuged to 5000 rpm during 15 minutes to

separate serum and stored frozen at -20°C until the analysis moment.

The serum concentrations of clindamycin were determined by the analysis of diffusion method on agar (9) with the strain of Staphylococcus aureus CDBJB - 1006 as test microorganism, and they were cultivated for 24 hours at 37°C with Mueller-Hinton agar for its growth.

The inhibition halos of the serum samples of the patients were measured to compare them with the standard drug. The standard curve of clindamycin was made with the following concentrations: (20, 10, 5, 2.5, 1.25, 0.625, 0.312 µg/mL), inhibition halos of the patients samples, and the standard curve were measured with an electronic digital caliper TRUPPER ®.

The concentration-time curves of the formulations (CLI-H-P, CLI-H-C y CLI) were graphed by a computer program (Software ORIGIN PRO 8.6 ®).

Pharmacokinetic Analysis:

The pharmacokinetic analysis was conducted using the computer program PKAnalyst ® (MicromathScientific Software, Salt Lake City, UT, USA).

The pharmacokinetic model that fitted best (R ≥ 0,95) to the three formulations was the model 3 of first order and one behavior, with the next formula:

Maximum Time of Concentration = (Dose*Kab)/(Volume Kab-Kelim) ⟦(e⟧^(-Kelim*Time))- (e^(-Kab*Time))

The pharmacokinetic variables obtained with the software were **K ½ ab**: absorption half-life, **K ½ el**: elimination half-life; Cmax: maximum plasma concentration; **Tmax**: time of maximum plasma concentration and **AUC**: area under the curve.

The variables obtained by mathematical formulas were:

% F: Percentage of bioavailability and Clb: Total depuration of the drug:

%F = ((AUC ⟦DOX⟧_x)/(AUC DOX-C))*100

Clb = Dose/AUC

Flip-flop kinetics can be demonstrated with the next formula (10):

Absorption rate = ⟦V_z (Kc+ΔC/Δt) ⟧_

Statics Analysis:

The normality of concentrations of the three formulations for each patient was calculated, through the ANOVA test. The data are presented in Table 1, as average ± standard error (average ± SE). Differences between the groups were obtained through the Tukey test.

Results:

The CLI-H-P formulation and CLI-H-C formulation show a higher value of **K½el, K½ab, Clb y %F** than the CLI formulation (table 1); however there is no difference between CLI-H-P and CLI. The **Tmax** value in the CLI-H-P, CLI-H-C y CLI formulations, was similar ($p > 0.05$).

Table 1: Mean ± 1 SD of pharmacokinetic variables for clindamycin after orally administration treated with 3 formulations: **CLI** (Control Clindamycin without excipients), **CLI-H-P** (Clindamycin + HPMC + Poloxamer 188) or **CLI-H-C** (Clindamycin + HPMC + Carbopol). The superscripts a, b and c indicate if there is significant difference between the 3 formulations ($p < 0.05$).

Pharmacokinetic Variables	CLI n = 3	CLI – H - P n = 9	CLI – H - C n=9
K ½ el (hrs)	6.33 ± 1.14 [a]	18.04 ± 1.08 [b]	19.10 ± 0.86 [b]
K ½ab (hrs)	2.17 ± 0.62 [a]	4.58 ± 0.98 [b]	4.82 ± 0.50 [b]
Clb (mL/min/Kg)	0.43 ± 0.01 [a]	0.09 ± 0.01 [b]	0.12 ± 0.01 [b]
%F (%)	100 [a]	461.94 ± 37.15 [b]	355.33 ± 24.13 [b]
Tmax (hrs)	2.70 ± 0.35 [a]	1.54 ± 0.29 [a]	2.35 ± 0.60 [a]
Cmax (µg*h/mL)	5.01 ± 0.31 [a]	8.10 ± 1.64 [b]	5.89 ± 0.84 [a]
AUC (µg*h)/mL	45.67 ± 3.51 [a]	210.97 ± 12.09 [b]	162.28 ± 26.0 [c]
AUMC (µg*h²/mL)	417.25 ± 29.62 [a]	5492.13 ± 4.02 [b]	4471.70 ± 21.88 [c]
AUC∞ (µg*h/mL)	50.87 ± 2.10 [a]	250.97 ± 5.62 [b]	186.27 ± 2.32 [c]

K½el (Elimination half-life), K½ab (Absorption half-life), Clb (Total depuration of the drug), F% (Bioavailability), Tmax (Time of Maximum Concentration), Cmax (Maximum Plasma Concentration), AUC (Area Under the Curve), AUMC (Area under the first moment of the concentration-time curve) and AUC∞ (Area under the concentration-time curve from zero to ∞ with extrapolation of the terminal phase).

There are significant differences between the three formulations for the variables **AUC**, AUMC y AUC∞, by comparing all against all.

During the medicine administration it was observed that the CLI-H-P formulation and CLI-H-C formulation reach detectable plasma concentrations up to 60 hours, unlike CLI-C with length only of 12 hours (Figure 1).

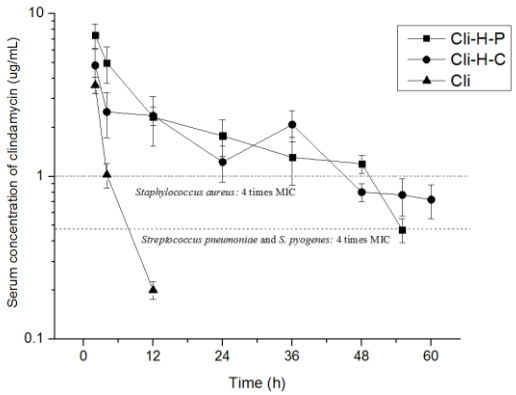

Figure 1: Mean ± 1 SD serum concentrations of clindamycin in dogs (n= 21) following oral administration of 20 mg/Kg of Two formulations of controlled release and compared with clindamycin without excipients. CLI : Clindamycin without excipients, CLI-H-P: Clindamycin + HPMC + Poloxámero 188, CLI-H-C. Clindamycin + HPMC + Carbopol.

Discussion:

The results of this study show that the addition of HPMC, poloxamer 188 and carbopol achieves clindamycin formulations of controlled release have a 48 hours effect; this is an innovative and valuable result in the efficiency of this active ingredient.

Additionally the mixes of different polymers with HPMC like carbopol have been used to produce supply systems of gastric retention in a controlled release formulation with success (7, 11).

There have not been reported studies about controlled release of clindamycin greater than 12 hours in dogs. As for human medicine have been reported studies of antimicrobial of controlled release in periodontics with mouthwashes, ointments and gels with active ingredients like chlorhexidine and metronidazole (12). The study of Norling (13) obtained a controlled release of 48 hours through a metronidazole formulation in suspension form. Also a study has reported a pharmaceutical invention in topical presentation of gel including an antibacterial agent (clindamycin) as treatment of vaginal infections in humans achieving controlled release up to 10 days (14).

The Cmax in the Saridomichelakis work was of 11 mg/Kg PO SID was of 3.812 ± 1.560 µg/g (15) comparing his values of work with this study, the Cmax was higher in the three formulations: 5.01 µg/g, 8.10 µg/g and 5.89 µg/g, for CLI, CLI-H-P and CLI-H-C respectively. The Cmax value of 4.4 µg/mL was determined in the study of Lavy (16) and the value was even lower by via IM in a dose of 10 mg/Kg SID than the formulations made in this study. In conclusion it can be mentioned that formulations CLI-H-P and CLI-H-C have a Cmax value higher due the use of HPMC that has properties allow controlling the release hydrophilic and hydrophobic drugs (17).

The pharmacokinetic value of Tmax in the present research it was found a shorter time to achieve Cmax, for CLI formulation was 2.70 hours, for CLI-H-P formulation was 1.54 hours and for CLI-H-C formulation was 2.35 hours. These values are only exceeded by parenteral via in the study of Lavy (16) with a dose of 10 mg/Kg, where the result was Tmax 73 by IM. This result is reasonable, because absorption of any active substance is faster and complete by parenteral via than enterally.

Regarding to AUC, in the study of Saridomichelakis (15) with a dose of 11 mg/Kg PO SID a value of 30.1 (µg*h)/mL was obtained; the values found in the present study were higher: 45.67 (µg*h)/mL, 210.97 (µg*h)/mL, 162.28 (µg*h)/mL, for formulations CLI, CLI-H-P and CLI-H-C respectively. Thus, the AUC value that determines the plasma concentrations of a drug after administration is higher in CLI-H-P and CLI-H-C formulations in function of time unlike the others authors. These values can be explained due the presence of the polymeric matrix they have mucoadhesive characteristics and its effect of controlled release (7). Even they exceed the values by parenteral via found by Lavy (16) where AUC was 24.3 hours for a dose of 10 mg/Kg by IV via, of 30.1 hours for IM via and 87.63 hours for via SC (16).

The Clb value in this study for the formulation CLI was: 0.433 mL/min/Kg, for the CLI-H-P was: 0.09 mL/min/Kg and for CLI-H-C was: 0.12 mL/min/Kg, however the study of Lavy (16) obtained a value greater than 6.10 mL/min/Kg. This Clb value is less in the CLI-H-P and CLI-H-C formulations due the characteristics of the excipients to produce systems of supply of gastric retention in a formulation of controlled release (17). A greater therapeutic time inside the organism is obtained and it is eliminated by mL/min/Kg slowly unlike values reported in the study of Lavy (16).

The %F found in the CLI-H-P formulation has a value of 116.93% and in the CLI-H-C formulation a value of 112.53%, unlike the study of Batzías (9), that obtained a smaller value of 72.55% with a dose of 11 mg/Kg PO SID. This values found of F% in CLI-H-P and CLI-H-C can be explained due that the HPMC is included in a polymer matrix which besides to confer controlled release, protects the active from degradation with acids and enzymes in stomach of dogs, increasing drug bioavailability (18). Carbopol is an agent of controlled release (7), besides, it increases the time of

gastrointestinal residence and improves the bioavailability of the drugs (9).

The formulations of controlled release of this research have reported that they have a pharmacokinetic of flip-flop type. According to Boxenbaum (10) the flip-flop condition for CLI-H-P and CLI-H-C can be demonstrated with the following formula:

Absorption Rate = ⟦V_z (Kc+ΔC/Δt) ⟧_

Where, for CLI-H-P the data of concentration-time at 24 and 60 hours are taken, ΔC/Δt = 0.018 µg/mL/h. and at the midpoint of this period of time (36 hours) (K)(C) = 0.9 µg/mL/h. In this way it is found that KC >> ΔC/Δt, therefore the absorption rate ≈ elimination rate; with this value it can be confirmed that exist a condition flip-flop for CLI-H-P and that in fact can be considered as a real extended release. Meanwhile for the CLI-H-C treatment, the following results were found: ΔC/Δt = 0.016 µg/mL/h and for (K) (C) = 0.2 µg/mL/h. thus KC >> ΔC/Δt, hence the absorption rate ≈ elimination rate; with this value as in CLI-H-P and CLI-H-C can be proved that exist a condition flip-flop, and that can be considered as a real extended release.

Among the benefits of drugs of controlled release, it can be included the maintaining of serum therapeutic concentrations for long periods of time, getting a longer time of drug effect, reducing the stress and patient handling during the drug administration, and achieving compliance of antimicrobial therapy (19). In this context as much CLI-H-P as CLI-H-C, were able to achieve serum therapeutic concentrations during 60 hours with a single dose orally.

According to the relation between Pharmacokinetic-Pharmacodynamic (PK/PD), clindamycin is a drug considered time-dependent, and it is bacteriostatic type to apply its action, due to this situation it achieves a better antimicrobial effect, and requiring to maintain the bacterial minimum inhibitory concentrations (MIC) for as long as possible (20). The results of pharmacokinetic variables of CLI-H-P and CLI-H-C got to improve the clinic efficacy of clindamycin, comparing it with presentations of immediate release.

It is worth mentioning that the microbiological method of agar diffusion used in this research to determine the serum concentrations of clindamycin, has been reported as sufficiently reliable and can replace the analytical results or conclusions derived from a liquid chromatography of high resolution (20). Above all, because the active fraction of drug or its efficacy is being determined, the microbiological technique of agar diffusion, offers more precise result and clinical conclusions than derivatives from a chromatography, which are purely chemical.

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