Volume-4, Issue-6, June-2015 • ISSN No 2277 - 8160

Research Paper | Vetinerary Science

Determination of Bioequivalence and Side Effects of Three Novel Rifaximin Isoforms in Dogs

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ABSTRACT

Objectives:Determine the bioequivalence of three novel variant of -rifaximin's -isoforms and the adverse effects produced by their administration.Material and method: 4 Beagle dogs were treated with a daily dose of 100mg / kg-1during 5 Days using a crossover design with aweek of purifying between them making the practices through spectrophotometrically light EUAV / Vis tests for plasmatic quantification of the antibiotic;the highestreached concentration was determined (Cmax), the maximum time to reach that concentration (Tmax) and the mean area under the curve according to the time AUC (0-t), as well as hematological, biochemical and fecal mucous cytology tests to detect possible modifications-in the renal and hepatic function and about intestinal microbiota, also monitoring the physical condition of each animal to determine symptomatology of side effects. Results and Conclusions:Our results show that, in the relationship between amorphous and alpha crystal batch there is a bioequivalence, but between them and the polymorphic alpha, there is not bioequivalence; the first two with an absorption and lower plasma concentrations and PC with greater absorption and plasma concentration. The Biochemical tests, and the absence of symptoms in the studied animals, showed non existence of side effects.

KEYWORDS : Rifaximin, bioequivalence, pharmacodynamics, isoforms, side effects.

INTRODUCTION

Rifaximin (C43H51N3O11) is practically insoluble in water but soluble in acetone and methanol with several active isoforms, is a semisynthetic product fermentation derived from rifamycin icaamycolaptosis(Streptomyces mediterranei), crystalline and hygroscopic orange-red color, (British Pharmacopoeia., 2012). It possesses antibiotic properties from ansamycins family, which derives of rifamycin S, with broad-spectrum bactericida and an action mechanism by inhibition of bacterial RNA synthesis (b subunit haloenzyme RNA polimerase dependent of DNA) without action on eukaryotic cells. Rifaximin is not inactivated by gastric acids and it isabsorbed in minimal amounts after oral administration, with a bioavailability lower than 0.4%, without being significantly affected by food. In human after an oral dose of 400 mg the highest plasma concentration which is reached at 1.2 hours is of 3.8 ng / ml; the mean area under the curve (AUC) was 18.35 ng × h / ml, with a half-life from 5.85 to 5.95 hours); 97% was recovered in feces and urine 0.32%, it was not detected in bile or milk (Gobernado and Ponce., 2004. Viscomi et al., 2008).

Five different crystalline forms of rifaximin(α, β, γ, δ ψ ε)have been identified and characterized by diffractometry of X ray on solid state, NMR and IR spectroscopy ATR-rays. The changes in the crystalline structure can produce differences from two to three orders of magnitude in the intrinsic dissolution rate, solubility and bioavailability of rifaximin. The bioavailability of the crystalline forms was assessed by quantifying of plasma level of rifaximin after the oral administration in dogs of each crystalline form; the Cmax, were distributed thought a range of three registers, the γ shape showed greater absorption and the β form the lowest. The ε and α forms have a bioavailability of approximately the same order of magnitudeas the β shape, while δ that has an intermediate absorption level between β and γ forms. In order to evaluate the dependent mechanism of the absorption dose, theγ form was administered to dogs at doses of 25, 100 and 300 kg mg / kg^{-1}. The results showed increases in absorption in dependent dose, but not in a lineal way, in the tested range. In the other hand, the δ shape and mainly the shape γ showed more similar bioavailability to the active absorbable ingredients (Viscomi et al., 2008).

The Rifaximin is not inactivated by gastric acids after oral administration and it is not significantly affected by food. The additional Pyridoimidazolering of rifaximin makes it practically non-absorbable by the cells of the gastrointestinal tract, (water insoluble) with a bioavailabil-

ity <0.4%, resulting in high concentrations of the drug in feces after oral administration (Eric L Brown et. al., 2010). The most common side effects in humans include abdominal pain, constipation, flatulence, bloating, fever, dizziness, headache, nausea, vomiting and diarrhea (J. Cave et. al., 2013).

MATERIAL AND METHODS

The study was conducted as published by Viscomi et al., 2008. Mariappana et al., 2004 Khamar&Satish., 2012. Lutfullah et al., 2011 and Divakar et al., 2012) with the following modifications : Calibration curves were established to spectrophotometry considering a blank of distilled water and blood plasma of control and issue samples, performing reading at 276 - 387nm (Khamar& Satish., 2012), by calculating the Cmax, Tmax and AUC o-24 hr.

Biological Material

The animals were obtained with permission and through vivarium "Claude Bernard" BUAP (BCB / 001/2012), supported and housed in the same place, according to the Mexican Official Standard NOM-062-ZOO-1999 "Technical Specifications for production, care and use of laboratory animals" and valued and secured its status as clinically healthy animal; complete clinical auscultation blood count, blood chemistry (glucose, renal and liver function), general urine and stool test (The minimum of animals was used, given the national and international restrictions on the use of live animals for experimentation).

Four Beagle dogs were dosed, with an average weight of 7 kg with free access to food during the day, overnight fast ingestion, and free access to water 24 hours, and as negative control two Beagle dogs of the same characteristics.

Material

Each isoform rifaximin drug (RIFA031007 and DRIFAC011304) were introduced into gelatin capsules and commercial product processing in tablet form.To each animal was administered an oral dose of 100mg / kg^{-1}.

The study was designed as cross-over kind and each dose was followed by a week as purifying period.

Methods

Blood samples were collected (2ml approximate) in tubes with EDTA as an anticoagulant.They were obtained by jugular venipuncture from each animal, at 1, 2, 4, 6, 8 and every 24 hours during 5 days; each sample was centrifuged at 3500 rpm for 5 minutes at room temperature to obtain the plasma.

The Rifaximin concentration was calculated using UV-Vis light spectrophotometry (EAUV-VIS) using a spectrophotometer brand Thermo model Genesis 10-Vis (Thermo Fisher Scientific Inc., Waltham, MA USA), the reading at 380 nm, from samples placed in plastic buckets of 1 or 2 ml and calculating the concentration using the formula: Absorbance of sample / Absorbance of standard X concentration of Standard.

The white solutions, the pattern, positive and negative control, and the issue in question were worked out in duplicate.

Standard curves made for comparison were: Rifaximin + plasm + H_2Od, rifaximin + Methanol and serum + H_2Od.

Data analysis was performed using the Microsoft Excel 2010 and Graph Pad Prism 5.0 software.

The blood cytometry analysis, renal function, liver function and fecal mucus cytology, were performed by a private laboratory of clinical analysis, with blood samples which were obtained every 24 hours throughout the duration of the trials.

RESULT AND DISCUSSION

Result

With the absolute values obtained, the spectrophotometric curves were performed to display respective pharmacodynamic trends. In the previous results, it was determined the mean and the SEM for time and for each group, the corresponding natural logarithm in order to standardize the information and thereby obtained a linear regression and the determination of Cmax, Tmax and ABC o-t was calculated.

The results of DRIFAC011304 show a slow absorption and plasma concentration whit aCmáx=32.578 mg/ml and Tmáx= ± 1hr (Table 1). The area under the curvewas 2.225mg h /ml (Graph No 1, Table No. 1a).

The results of RIFA031007, show a slow absorption and plasma concentration, whit a Cmáx = 23.202mg/ml and Tmáx = ± 1hr (Table 2). The area under the curve for these was 2.216mg h /ml. (Image No 2. Table No. 2a).

The results of P. C, show a low absorption and plasma concentration, whit a Cmáx = 23.202 mg/ml and Tmáx = ± 1hr (Table 3). The area Under the Curve was 2.216 mg/ml (Graph No 3. Table No. 3a).

The laboratory studies tests were analyzed qualitatively; hematological cytometry showed variations within normal physiological parameters during the experimental time. Clinical blood chemistry tests for kidney function (urea, creatinine, BUN, protein) and for liver function (ALT / TGS, protein, albumin) were maintained in physiological values at all times. General urine tests did not reported any evidence of alteration.

In the results of fecal mucus cytology were performed in order to evaluate qualitatively the intestinal microflora of the testing animal, they reported increase in the presence of Gramm-negative bacteria during treatment with RIFA031007.

Clinical side effects are not present, with the exception of modification fecal matter consistency (paste) for RIFA031007 administration, without reaching diarrhea.

DISCUSSION

NOM-177-SSA1-1998, establishes in section 9. 1. 5. Accuracy: "the average value of the measurements at each concentration level of data repeatability and reproducibility should be within ± 15% of nominal concentration, except the RIA methods that should be within 20%.For a variety of drugs, a range of 80-120% for the ratio of the averages of products is enough as a equivalence criterions, when the survey data are analyzed on the original scale. This corresponds to a range of ± 20% to the relative difference between the averages of the products. When the data is transformed to its logarithm using the AUC and Cmax analysis as equivalence criterion, interval 80 to125% It is used a ratio of the averages".

Considering the previous information; our results show that, in the relationship between RIFA and DRIFAC there is a bioequivalence and between them and the PC, do not exists any bioequivalence. The first, with absorption and plasmatic lower concentrations, and the PC, with absorption and greater plasma concentration.

Regarding side effects, in no case symptomatic or biochemical changes suggestive of adverse effects were occurred.

Modifications concerning to increasing bacterial presence in feces, which agree with the reported by Department of Health and Ageing, Therapeutic Goods Administration (TGA) 2012 who awarded it to: 1) modification of the intestinal microbiota or 2) a change in the environmental conditions in the intestinal lumen of the host.

CONCLUSSION

Rifaximin is an antibiotic useful in gastrointestinal-infections in their amorphous and alpha isoforms and systemic infections in polymorphic form, given their bioavailability differences and not alter hepatic and renal function.The microbiological changes in fecal mucus, except the consistency and color of faecesproduced no modification in nutritional status of the animals.

FINANCING

The project was funded and chemicals provided by Interquim, SA de CV, Mexico.

ACKNOWLEDGMENTS

We appreciate the collaboration of the owners and staff of the Laboratory of Clinical Analysis, "Asuncion" Tecamachalco, Puebla.

CONFLICT OF INTEREST

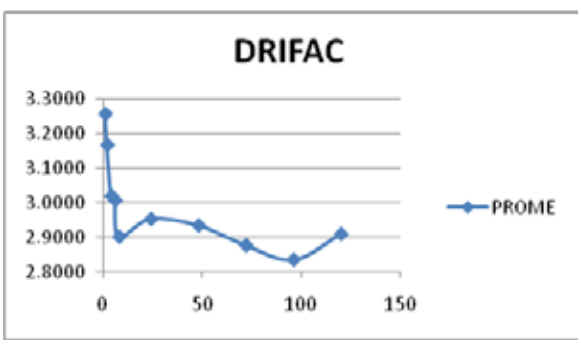
There are no conflicts of interest for any author.

Table No. 1. Results of DRIFAC011304; P (1-4) = Number of dog, µ/ml = values microgram/ml, Ln=Natural logarithm.

Hr	P1 µ/ml	P2 µ/ml	P3 µ/ml	P4 µ/ml	P1 Ln µ/ml	P2 Ln µ/ml	P3 Ln µ/ml	P4 Ln µ/ml	PROME
1	2171	1128	1749	2533	3.33665982	3.0484438	3.24278981	3.40329215	3.2578
2	1341	929	1967	1910	3.12742878	2.96801571	3.29380436	3.28103337	3.1676
4	1246	806	896	1327	3.09551804	2.90633504	2.95230801	3.12287092	3.0193
6	1171	891	872	1171	3.0685569	2.9498777	2.94051648	3.0685569	3.0065
8	943	701	616	981	2.97451169	2.84571802	2.78958071	2.99156901	2.9004
24	815	664	801	1483	2.91135761	2.82216808	2.90368252	3.37134115	2.9520
48	720	799	798	1398	2.8579325	2.86854444	2.86332286	3.14550717	2.9337
72	801	780	782	697	2.90368252	2.86332286	2.89320675	2.84828378	2.8758
96	602	682	654	810	2.77959649	2.83378437	2.81557775	2.90848502	2.8344
120	602	1768	521	777	2.77959649	3.24748226	2.71683772	2.89043102	2.9086

DRIFAC011304.AUC 2.225 µg h/ml.

Table No. 1. Results of DRIFAC011304; P (1-4) = Number of dog, µ/ml = values microgram/ml, Ln=Natural logarithm.



AUC	Log
Baseline	0.0
Total Area	267
Total PeakArea	267
Number of Peaks	1.0
Peak 1	
First X=	1.0
Last X=	120
Peak X=	1.0
Peak Y=	2.3
Area=	267
%Area=	100

Hr	P1 µg/ml	P2 µg/ml	p3 µg/ml	P4 µg/ml	P1 Ln µg/ml	P2 Ln µg/ml	p3 Ln µg/ml	P4 Ln µg/ml	PROME
1	192	195	212	241	2.28330123	2.27003461	2.32569878	2.38201704	2.32016192
2	160	182	200	179	2.20411998	2.26007139	2.30164865	2.25285303	2.25467316
4	179	160	171	187	2.25285303	2.20411998	2.23315357	2.27184161	2.24049205
6	160	149	170	160	2.20411998	2.17318627	2.25134013	2.20411998	2.20519162
8	150	141	153	155	2.17509126	2.14921911	2.1833561	2.1903317	2.17474954
24	197	187	203	195	2.29446523	2.27184161	2.30778719	2.27148394	2.27189474
48	170	212	170	185	2.23044892	2.32603586	2.23134023	2.26737173	2.26382419
72	195	167	192	168	2.29003461	2.22271647	2.28269461	2.22530928	2.25518874
96	174	172	195	165	2.24054925	2.23552845	2.29069189	2.21748394	2.24606363
120	163	139	202	155	2.21218876	2.13353891	2.30626967	2.1903317	2.21057972

Table No. 2. P (1-4) = Number of dog, µ/ml= values in microgram/ml, Ln=Natural logarithm.

AUC	Log
Baseline	0.0
Total Area	266
Total PeakArea	266
Number of Peaks	1.0
Peak 1	
First X=	1.0
Last X=	120
Peak X=	1.0
Peak Y=	2.3
Area=	266
%Area=	100

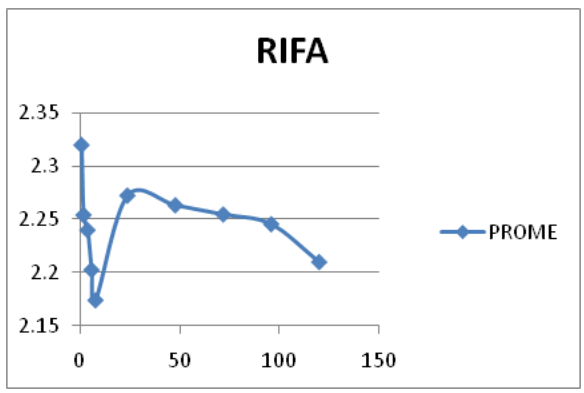
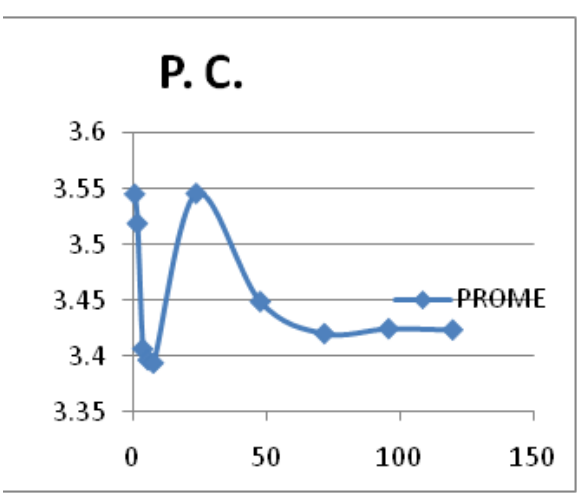


Image No.2, and Table No. 2a. Lineal regression (concentration/time) of the drug RIFA031007 AUC=2.216 µg h/ml.

Hr	P1 µ/ml	P2 µ/ml	P3 µ/ml	P4 µ/ml	P1 Ln µ/ml	P2 Ln µ/ml	P3 Ln µ/ml	P4 Ln µ/ml	PROME
1	3661	3394	3139	3603	3.56356979	3.53071184	3.49679132	3.59139855	3.54562536
2	3394	3539	3018	3297	3.53071184	3.54888056	3.47971924	3.51811895	3.51935765
4	2579	2309	2885	2279	3.40993312	3.39550066	3.46014582	3.35774433	3.40683098
6	2255	2910	2727	2145	3.35314655	3.46730206	3.43598514	3.3314273	3.39689276
8	2455	2315	2861	2339	3.38667728	3.364551	3.45651785	3.36903012	3.39419409
24	4206	3515	3418	3030	3.62386927	3.54592533	3.53377236	3.48144263	3.54625232
48	2594	2703	2836	3152	3.41396997	3.43184605	3.45270623	3.49858621	3.44927733
72	2727	2594	2764	2461	3.43568514	3.41396997	3.44153834	3.39111161	3.42057639
96	2536	2873	2861	2339	3.41597441	3.45833563	3.45651785	3.36903012	3.42486453
120	2764	2824	2509	2533	3.44153804	3.45086469	3.39953066	3.40363519	3.42388465

Table No. 3. P (1-4) = Number of dog, µ/ml= values in microgram/ml, Ln=Natural logarithm.



AUC	Log
Baseline	0.0
Total Area	410
Total PeakArea	410
Number of Peaks	1.0
Peak 1	
First X=	1.0
Last X=	120
Peak X=	24
Peak Y=	3.5
Area=	410
%Area=	100

Image No.3, and Table No. 3a. Lineal regression (concentration/time) of the drug P. C. AUC=3.416 μg h/ml.

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