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Evaluation of bioequivalence of two tylosin formulations after oral administration in broiler chickens

Ocena biorównoważności pomiędzy dwoma preparatami tylozyny
po podaniu doustnym u kurcząt rzeźnych

SUMMARY

Tylosin, a drug mainly used in veterinary medicine, belongs to the macrolide group of antibiotics. In this study the bioequivalence and differences in pharmacokinetics of solution and powdered tylosin formulations were established. The following recommended by EMEA parameters: C_{max}, T_{max}, AUC were determined. All the investigations were carried out in the populations of 74 broilers chickens. The concentration of tylosin in plasma was determined by means of the appropriately modified HPLC method. The mean maximum concentration (C_{max}) of tylosin in plasma was found to occur 3.0 h (for powder and liquid) after oral administration. They were 403.20 ng/ml and 403.12 ng/ml respectively. The plasma profiles of tylosin following administration of both formulations were similar.

Key words: tylosin, oral administration, bioequivalence, chickens

INTRODUCTION

Tylosin, a drug mainly used in veterinary medicine, belongs to the 16-member-ring macrolides group of antibiotics. It inhibits bacterial protein synthesis by blocking the translocation step [Brisson-Noel *et al.* 1988]. This chemotherapeutic is a lipid-soluble organic base with 40% binding to serum proteins, a high degree of lipid solubility and a low degree of ionization. It is thus widely distributed throughout tissues and body fluids [Gingerich *et al.* 1977]. Tylosin is active against mycoplasmas, anaerobic bacteria and Gram-positive bacteria [Prescott and Baggot 1988; Prats *et al.* 2002]. It is extensively used for the treatment of pneumonia, arthritis, CRD and other infections caused by susceptible organisms [Taha *et al.* 1999]. Pharmacokinetics studies of tylosin have been reported in cows, calves, goats and dogs [Prats *et al.* 2002]. The pharmacokinetics of tylosin in poultry are poorly documented. In this study the bioequivalence and differences in pharmacokinetics of solution and powdered tylosin formulations were established. The following recommended by EMEA parameters: C_{max}, T_{max}, AUC were determined.

Bioequivalence studies are designed to examine whether the systemic bioavailability of a test product and those of the reference product differ significantly. Following the relevant FDA and EMEA Guidelines, the statistical analysis should be based on the non-compartmental parameters C_{max}, T_{max}, AUC, derived from the drug concentration-time curve (although plasma is a preferred matrix, sometimes whole blood are used).

MATERIALS AND METHOD

Chemicals and reagents

The tylosin standard (INN-Tylosin tartate) was supplied by Sigma-Aldrich (St Louis, USA). HPLC-grade acetonitryle and methanol were obtained from Becker (Darmstadt, Germany). potassium dihydrogen phosphate and orthophosphoric acid 85% were obtained from POCH (Gliwice, Poland), trichloroacetic acid was obtained from Merck (Phillipsburg, USA). All other reagents were of analytical grade.

Apparatus

The chromatographic system used was a Varian liquid chromatograph (Varian, USA). It consisted of a solvent delivery pump (STAR 9002), a 10 µL volume manual injector, a variable wavelength UV-VIS detector (all Varian Analytical Instruments, USA). Chromatographic separations were performed using a Merck LiChroCART 125×4 mm, PuroSpher RP-18C column (5 µm particle size). The chromatograms were taken directly from the program Varian Star Chromatography Workstation Version 4.51. A centrifuge (MPW 210), an analytical balance (Sartorius BP 61S), cartridges C18, 500mg (Shimadzu) were also used.

Drugs

Oral administration was performed using Tylosina 20% liquido (Chemifarma) and Tylan Soluble powder (Eli Lily Elanco), which is marketed as solution and powder, respectively. The active substance was administrated at dose of 20 mg/kg b.w. The dose was chosen according the manufacturers' instruction.

Animals

Seventy four, both sexes, Hubart Evolution broiler chickens weighing 1600–2140 g, were used in the study. The animals were purchased from a poultry farm RSP Wola Przybysławska. The chickens were housed in individual pens. The animals were fed standard laboratory food without chemotherapeutics and coccidiostatics and were given water *ad libitum*. The subjects were all in a good health, as determined by the history, physical examination and hematological tests. The animals were randomly assigned to two groups (A and B). In this study chicken from group A were administrated a dose of 20 mg of tylosin/kg b.w. of Tylosina 20% liquido by the oral route. Group B were treated with Tylan Soluble powder at the same dose and by the same route. Blood samples were collected from the wing vein in heparinised tubes at time 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0 hour after dosing. Plasma was separated by centrifugation at 3500 g for 15 minutes and stored at -30°C until assayed.

METHOD

0.5 ml 0.2 M K_2HPO_4 (pH = 9) was added to 1 ml of plasma. The mixture was agitated and next deproteinated with 1 ml of 5% trichloroacetic acid. The mixture was centrifuged at 5500 g for 15 minutes. The supernatant fluid was diluted with 0.2 M phosphoric buffer (pH = 9) to 24 ml

volume. The mixture was cleaned and thickened with solid-phase extraction (SPE) with C_{18} cartridge. The cartridges were activated with 10 ml of methanol and conditioned with 10 ml 0.1 M phosphoric buffer (pH = 8). Finally, the cartridges were cleaned with 5 ml phosphoric buffer (pH = 8) and dried. Dry residues with absorbed tylosin were eluted from cartridges with 0.5 ml acetonitrile and analysed by HPLC. Plasma concentration of tylosin was determined by means of the appropriately modified high performance liquid chromatography (HPLC) method [Prats *et al.* 2002].

The mobile phase was composed of acetonitrile and 0.04 M KH_2PO_4 with pH adjusted to 2.6 (30:70 v/v/v). The mobile phase was pumped isocratically at a flow rate of 1 ml/min. The variable wavelength UV detector was set at 280 nm. All analyses were performed at ambient temperature. The assay was validated by measuring the concentration of known amounts of tylosin in chicken plasma. The linearity, precision, accuracy and specificity were calculated (n = 6). The mean recovery of tylosin from plasma samples was 91.5±1.89%. The detection limit and limit of quantification were 20.6 ng/ml and 68.5 ng/ml, respectively.

DATA ANALYSIS

The concentration of drugs vs. time curves for each individual animal were analysed, with non-compartmental analysis based on statistical moment theory using PK Solutions 2.0 computer program. The area under plasma concentration-time curve (AUC) was calculated by the trapezoidal method from time zero to the last sample. For peak plasma tylosine concentration (C_{max}) and the time to peak concentration in plasma (t_{max}) observed values were taken. All values are reported as mean ±SD. In order to verify whether there were significant statistical differences between the two formulations studied, an ANOVA test was applied as a prior step for AUC, t_{max} and C_{max} parameters. Bioequivalence was conducted when these parameters fell within the limits of 0.8–1.20 [Toutain and Koritz 1997]. In general, the confidence interval for untransformed data should be 80–120% (the confidence interval should lie within ±20% of the mean of the reference product). For logarithmically transformed data, the confidence interval is generally 80–125% (the confidence interval should lie within -20% +25% of the mean of the reference product).

RESULTS AND DISCUSSIONS

The mean plasma concentration-times curves following oral administration of both tylosin formulations are presented in Fig. 1. The pharmacokinetics parameters calculated for each formulation is summarized in Tab. 1. The plasma profiles of tylosin following the administration of both formulations were similar. Both formulations demonstrated that they were absorbed progressively. After oral dosing Tylosina 20% liquido and Tylan Soluble powder the peak serum concentration (403.0±7.33 and 403.26±11.35 ng/ml, respectively)

Table 1. Pharmacokinetics parameters (mean ±SD) calculated by non-compartmental analysis after oral administration of two formulation of tylosin (20 mg/kg b.w.) to chicken

Tabela 1. Parametry farmakokinetyczne (średnia ±SD) wyznaczone metodą niekompartmentową po doustnym podaniu dwóch preparatów tylozyny (20 mg/kg m.c.) kurczętom

Parameters	Tilosina 20% liquido	Tylan Soluble powder
C_{max} (ng/ml)	403.12±7.22	403.2±11.33
AUC (ng·h/ml)	2303.5±99.5	2223.1±114.5
T_{max} (h)	3.0	3.0

were attained at 3.0 hours in all animals. The area under the plasma concentration-time curve (AUC) were 2303.5 and 2223.1 ng · h/ml for solution and powder, respectively. Plasma levels of tylosin decreased slowly and the drug was still detectable in high concentration (above MIC) at 8 hours.
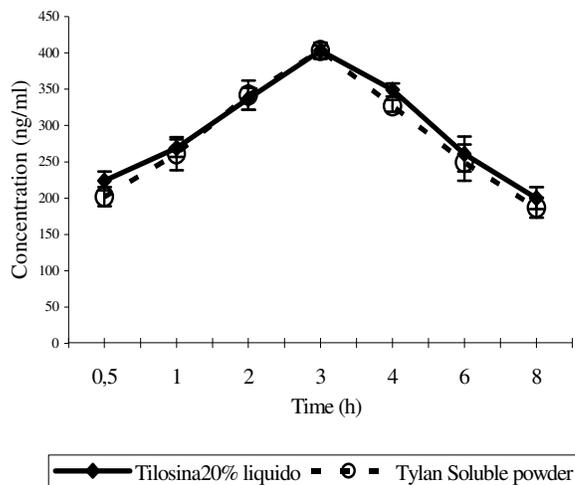

Fig. 1. Mean plasma tylosin concentration in chicken after oral administration of two formulation of tylosin (20 mg/kg b.w.)
Fig. 1. Średnie stężenie tylozyny w plazmie kurcząt po doustnym podaniu dwóch preparatów zawierających tylozynę (20 mg/kg m.c.)

The comparative analysis of the kinetic parameters of the two formulations showed no statistically significant differences in AUC, C_{max} and t_{max} (Tab. 2). This data conform with the bioequivalence criteria and it is possible to conclude that the two formulations are equivalent.

Table 2. Evaluation of bioequivalence of two formulations with tylosin in chickens
Tabela 2. Ocena biorównoważności dwóch preparatów tylozyny u kurcząt

Parameters	Tilosina 20% liquido	Tylan Soluble powder	Differences in percent ($\pm$ 20 %)	ANOVA
C_{max} (ng/ml)	2303.5	2223.1	3.59	+1.03
T_{max} (h)	403.12	403.2	0.1	-0.99
AUC (ng-h/ml)	3.0	3.0	0.0	1.0

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STRESZCZENIE

Tylozyna, używana głównie w medycynie weterynaryjnej, należy do antybiotyków makrolidowych. W wykonanych badaniach ustalono stopień biorównoważności i zakres różnic w farmakokinetyce pomiędzy preparatami zawierającymi tylozynę stosowanymi w postaci proszku i roztworu. W trakcie badania oznaczono wskaźniki zalecane przez EMEA: C_{max}, T_{max}, AUC. Badania przeprowadzono na 74 kurczętach rzeźnych. Do oznaczania tylozyny w osoczu kurcząt wykorzystano metodę HPLC w modyfikacji własnej. Średnie maksymalne stężenia tylozyny w plazmie stwierdzono po 3 h (dla proszku i roztworu) od podania doustnego i wynosiły one odpowiednio 403,20 i 403,12 ng/ml. Profil stężeń tylozyny w plazmie po podaniu obu preparatów był bardzo podobny.

Słowa kluczowe: tylozyna, podanie doustne, biorównoważność, kurczęta