EVALUATION OF THE BIOEQUIVALENCE OF TWO ERYTHROMYCIN THIOCYANATE FORMULATIONS AFTER ORAL ADMINISTRATION TO BROILER CHICKENS

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Abstract

Twenty-one eight-week-old healthy broiler chickens of both sexes were used in the experiment. The birds were randomly allotted to three groups: two experimental (A and B) and one control. The chickens from group A received erythromycin in the form of granulate (Erytrowet® granulat), while the chickens from group B received erythromycin as a powder (Erytrowet®). Both formulations were administrated orally at a single dose of 25 mg/kg b.w. Blood samples were collected at 0.5, 1, 2, 2.5, 3, 4, 6, 8 and 12 h after administration of the drugs. Erythromycin concentrations were determined by the HPLC method. Comparison of the plasma pharmacokinetic profiles of both products indicated that there were no differences in the basic pharmacokinetic parameters between both formulations. The results indicate that the formulations used in this study are bioequivalent.

Key words: chickens, erythromycin thiocyanate, pharmacokinetics, drug formulation.

Macrolides are a large group of antibiotics, which naturally occur in the environment (2, 3). The great advantage of this group of chemotherapeutics is its excellent penetration into cells (especially macrophages) and tissues, despite relatively low concentration in serum. Macrolides have an affinity to the lungs, liver, kidneys, spleen, and reproductive tract (2, 4). The concentration of the drugs in the peripheral compartment is five to ten-folds higher than in the central compartment. Macrolides are not sensitive to β-lactamase (6), so they are an alternative to penicillins and cephalosporines. Among the adverse effects of macrolides we can include diarrhoea, nausea, and vomiting (1, 6).

In veterinary medicine, antibiotics from this group are widely used in the treatment of pneumonia, mastitis, and chronic mycoplasmal infections of the respiratory tract (2). Erythromycin is a macrocyclic compound containing a 14-membered lactone ring with ten asymmetric centres and two sugars (L-cladinose and D-desoamine), making it a compound very difficult to receive via synthetic methods. It is produced from a strain of the actinomycetes - Saccaropolyspora erythraea, formerly known as Streptomyces erythraeus. This antibiotic prevents bacteria from growing by interfering with their protein synthesis. Erythromycin binds to the 23S rRNA molecule in the 50S of the bacterial ribosome, blocking the exit of the growing peptide chain, thus inhibiting the translocation of peptides (4). In veterinary medicine, erythromycin is used mainly as a stearate, ethylsuccinate, lactobionate, or base. According to our knowledge there is no data about the pharmacokinetic properties of erythromycin thiocyanate in chickens.

The aim of the present study was to investigate and compare the basic pharmacokinetic parameters of erythromycin thiocyanate administrated orally in broiler chickens with the use of two commercial preparations.

Material and Methods

Drugs, chemicals, and reagents. Erythromycin standard (E C. No. 204-040-1) was purchased from Sigma (USA). Acetonitrile (ACN) and methanol were of chromatography grade and they were purchased from Merck (Germany). Other chemicals: phosphate acid 85%, citrate acid monohydrate, methylene chloride (CH$_2$Cl$_2$), hydrochloric acid, dipotassium hydrogenophosphate trihydrate (K$_2$HPO$_4$·3H$_2$O), potassium dihydrogenophosphate (KH$_2$PO$_4$), and NaOH. These were of analytical grade and they were from POCH (Poland). HPLC grade water was produced by means of a Milli-Q-Plus 185 system (Millipore, France).

Apparatus. Analyses were preformed with a gradient liquid chromatography (Gilson, USA), and a
fluorometric detector RF-551 (Shimadzu, Japan). Compounds were separated on a Lichrocart 125-3 Purospher RP-18e 5µm column (Merck, Germany). A SPE clean-up steps were done with aromatic sulfonic acid (C₆H₅SO₃H) in solid-phase extraction cartridges (J.T. Baker, The Netherlands).

**Animals.** The study was performed on 21 eight-week-old, healthy broiler chickens of both sexes (11 males and 10 females). All chickens were obtained from a poultry-breeding farm. Before commencing the study, the chickens were marked with numbers. The chickens were allowed a 7-d acclimatisation period prior to the study. Feed (antibiotic-free commercial diet) and water were given ad libitum throughout the study. The local Ethical Commission of the University approved all procedures involved in the study. The birds were randomly allotted to three groups. Group A and B were used to investigate the pharmacokinetic parameters of erythromycin. The chickens from the group A received the antibiotic in the form of granulate (Erytrowet® granulat, TZF Polfa Poland), while the chickens from group B received erythromycin in the form of powder (Erytrowet®, TZF Polfa Poland). The third untreated group (K) was used to obtain control plasma. Both formulations were administrated orally in a single dose of 25 mg/kg b.w. The antibiotic was administrated individually by a gavage. For the simplification of drugs application, the required individual dose of both drugs was mixed with a little volume of water to form pulp or pulp with small solid granules and immediately administrated directly into the crop using a plastic tube attached to a syringe. Finally, the remains of granules and pulp were washed down with approximately 2-3 ml of drinking water.

Blood samples were collected from the brachial veins of each chicken into heparinised tubes at 0.5, 1, 2, 2.5, 3, 4, 6, 8, and 12 h after administration of the drugs. The plasma was separated and stored at -30°C until the day of analysis.

**Chromatographic procedure.** The plasma concentration of erythromycin was quantified with the use of high-performance liquid chromatography (HPLC) according to the method of Dreassi et al. (6) with our own modification (changes in the flow rate and gradient flow). To our best knowledge, to date this method was used to measure erythromycin in meat, fish, kidneys, liver, and milk, but not in plasma. A mobile phase was composed of HPLC-eluent A – 0.03 M phosphate buffer, pH 7.0 /ACN 36:64 v/v, and HPLC-eluent B – ACN. The flow rate was fixed at 1.1 mL/min. The gradient was initiated during 10 min with 100% eluent A followed by an increase to 25% eluent B over 5 min, and 100% eluent A for the next 5 min. The fluorometric detection was done with a 260 nm excitation and a 305 nm emission wavelengths. All analyses were performed at ambient temperature.

**Statistical analysis.** The statistical analysis was performed using the Statistica 6.0 (Stat Soft Inc, Tulsa, Oklahoma, USA). The differences between the plasma pharmacokinetic parameters of chickens from groups A and B were determined by one–way ANOVA. A value of  P≤0.05 was considered significant. The pharmacokinetic parameters are reported as mean ± SD.

**Results**

The method was validated by the determination of linearity, precision and accuracy, recovery, limit of detection, and limit of quantification. Linearity was determined by generating the calibration curve. For the construction of the curve, five standard concentrations of erythromycin in the range of 0.5 to 6 µg/mL were prepared. The correlation coefficient (R²) was equal to 0.99907. The limit of detection was 0.240 µg/mL and the limit of quantification 0.801 µg/mL. The precision of the method was determined by repeatability. The relative standard deviation was ≤1.39%. The accuracy was represented by recovery. The mean recovery of erythromycin from plasma samples was 97.50% ± 0.83.

Pharmacokinetic parameters were calculated using classic equations (9). Table 1 shows the basic pharmacokinetic parameters calculated after oral dosing with the help of the “PK Solution 2.0” computer program. A non-compartmental model is often used in the calculation of pharmacokinetic parameters especially in bioequivalence studies (12, 13). In addition, the mean plasma concentrations after oral administration of both formulations are compared in Fig. 1.
concentrations (Cmax) and times to peak concentrations represents the elimination rate constant. The peak time (MRT) was calculated using trapezoid area calculations extrapolated to infinity. The mean residence zero to the last time with a measurable concentration, AUMC∞→t for chickens from Group A they were from 6.8 to 10.8 g·h/mL. The values of Cmax in each individual animal was matched to the "PK Solutions 2.0" computer program. The elimination half-life (t1/2el) was calculated as \( t_{1/2el} = \frac{\ln 2}{\lambda z} \), where \( \lambda z \) represents the elimination rate constant. The peak concentrations (Cmax) and times to peak concentrations were read from the plotted concentration-time curve of each drug in each individual animal. The area under the plasma concentration-time curves (AUC 0→t) from time zero to the last time with a measurable concentration was calculated by trapezoidal rule. The mean residence time (MRT) was calculated using trapezoid area calculations extrapolated to infinity.

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MRT = \frac{\text{AUMC}_0}{\text{AUC}_0}\]

where AUMC∞ is the total AUMC computed using exponential terms.

The values of AUC 0→t for chickens from Group B were from 6.2 to 10.2 \( \mu \)g·h/mL and for chickens from Group A they were from 6.8 to 10.8 \( \mu \)g·h/mL. The values of Cmax in chickens receiving Erytrowet® were insignificant and achieved values from 2.4 to 3.1 \( \mu \)g/mL. In the second group receiving Erytrowet® granulat, the values of Cmax were from 2.4 to 3.5 \( \mu \)g/mL. In both cases the Cmax values were lower than the values reported by Goudah et al. (10). In the previous study the erythromycin was given (in solution in the previous study, as a pulp in this study). The elimination half-lives (2.01 ±0.20 h – powder and 1.90 ±0.63 h - granulate) were much shorter compared with the values observed in the previous study (4.1 h, (10). The MRT values (3.26 ±0.32 h – powder and 3.56 ±0.36 h - granulate) were almost similar to the values reported by Goudah et al. (10). The plasma pharmacokinetic profiles obtained following oral administration of both formulations were similar. The results of our study indicate that there are no statistically-significant differences between both investigated formulations. No significant differences were found for the basic pharmacokinetic parameters Cmax, tmax, t1/2, AUC0→t and MRT. This data points to similar pharmacokinetic properties of both formulations.

At present, a lot of pharmaceutical manufacturers produce veterinary drugs in granulate form. The main reason for granulation is the protection of its constituents from the segregation, which usually occur when we use powder, and more regular distribution of drugs in feed. Granulate is characterised by longer-term usage and because it is denser than the parent powder it occupies less volume per unit weight. Granulate is therefore more convenient for storage and shipment.

In conclusion, the present study indicated that drug formulation did not influence significantly the basic pharmacokinetic parameters of erythromycin after oral administration to broiler chickens as a pulp and as a pulp with little granules. Products used in this study could be considered as bioequivalent.

Table 1

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Erytrowet®</th>
<th>Erytrowet® granulat</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>t1/2el (h)</td>
<td>2.01 ± 1.20</td>
<td>1.90 ± 0.63</td>
<td>ns</td>
</tr>
<tr>
<td>Cmax (µg/mL)</td>
<td>2.76 ± 0.29</td>
<td>3.06 ± 0.41</td>
<td>ns</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>2.50 ± 0.50</td>
<td>2.50 ± 0.50</td>
<td>ns</td>
</tr>
<tr>
<td>AUC0→t (µg·h/mL)</td>
<td>7.68 ± 1.54</td>
<td>8.66 ± 1.55</td>
<td>ns</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>3.26 ± 0.32</td>
<td>3.56 ± 0.36</td>
<td>ns</td>
</tr>
</tbody>
</table>

\(t_{1/2el}\) – elimination half life, \(C_{max}\) plasma maximum concentration, \(t_{max}\) time of maximum concentration, \(AUC_{0\rightarrow t}\) area under the plasma concentration vs. time from time 0 to infinity, MRT mean residence time, ns – non-significant

Discussion

The pharmacokinetics of erythromycin has been studied on calves (5), rats (11), foals (13), goats (1), horses (8), pigeons (15), but there is only one article about the pharmacokinetics of erythromycin in chickens. Goudah et al. (10) used microbiological assay to investigate erythromycin concentration in chickens' plasma, but they applied drug which contained 10% erythromycin free base. In our study, plasma pharmacokinetics profiles for erythromycin after oral administration of both formulations were almost identical and reached 2.5 h. This was a higher value compared with the earlier report on broiler chickens (1.3 h) (10). This difference is the consequence of the form in which the erythromycin was given (in solution in the previous study, as a pulp in this study). The elimination half-lives (2.01 ±1.20 h – powder and 1.90 ±0.63 h - granulate) were much shorter compared with the values observed in the previous study (4.1 h, (10). The MRT values (3.26 ±0.32 h – powder and 3.56 ±0.36 h - granulate) were almost similar to the values reported by Goudah et al. (10). The plasma pharmacokinetic profiles obtained following oral administration of both formulations were similar. The results of our study indicate that there are no statistically-significant differences between both investigated formulations. No significant differences were found for the basic pharmacokinetic parameters Cmax, tmax, t1/2, AUC0→t and MRT. This data points to similar pharmacokinetic properties of both formulations.

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References