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SHORT COMMUNICATION

PHARMACOKINETICS OF COLISTIN IN CHICKENS AT DIFFERENT AGES

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Colistin is a decapeptide antibiotic with a narrow spectrum of antibacterial activity mainly against Gram-negative microorganisms (Ziv,1981). It is also able to inactivate bacterial toxins *in vitro* (Ziv *et al.*, 1978). That is why it is commonly used for treatment of diseases caused by sensitive Gram-negative bacteria (Roy *et al.*, 1997; van Hattum *et al.*, 2000).Despite the fact that this antibiotic is used for many years, the investigations on its pharmacokinetics in poultry are limited. The data about its oral absorption are contradictory. The lack or the very low values of absorption are well known (Roudaut, 1989). However, there are also data showing significant blood levels after oral (p.o.) administration in broiler chickens (Lashev and Haritova, 2003), and its absorption is probably influenced by the age.

The data from the literature show that the age of chickens can influence the behaviour in the organism of several antibacterial drugs, although the reported results are contradictory (Pashov, 1983; Pashov and Kanelov, 1994; Santos *et al.*, 1996; Lashev, 2000).

The aim of the present study was to investigate the pharmacokinetics of colistin in chickens at different ages.

Colistin sulfate (21200 UI = 1 mg), batch No 9498, was provided by Zavet Ltd, Zavet, Bulgaria.The antibiotic was used as a water solution for i.v. administration. A drug formulation Colistin-pulvis (containing 120 000 000 UI colistin sulfate and excipientes ad 100.0), provided by Zavet Ltd, was used for oral administration.

Experiments were carried out on 178 chikens (*Gallus domesticus,* breed Decalb) at the age of 1 week, 1 month and 2 monthd from both genders, weighing 50.88 ± 0.68 g, 204.0 ± 1.2 g and 1068.0 ± 4.0 g, respectively. Six one–year old hens (*Gallus domesticus,* breed Decalb) weighing 1840 ± 5.0 g were also included in the experiments. The chickens and hens were kept in cages (six birds in a cage) and given free access to food (commercial antibiotic free diet) and water. Two experiments were carried out:

Experiment I

In the first experiment 85 birds were included. They received i.v. colistin sulfate as water solutions in the cutaneous ulnar vein. Colistin was administered to chickens and hens at a dose of 1 mg/kg as 0.15–0.6 % water solutions, according to the age of the treated groups. The blood samples were collected from the contralateral vein, immediately prior to drug

administration and at 0.083, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5 and 7 post treatment hours. The 7-day old chickens were allocated in 11 groups, each group consisting of 5 animals. Blood samples were collected from one group at each interval after euthanasia of the animals. The 1-month-old chickens were grouped in 4 groups. Group 1 (n=3) was untreated and sampled at 0 h. Groups 2, 3 and 4 (5 birds each) were treated and sampled as follows: the second group – at 0.083, 0.75 and 2 hours; the third group – at 0.5, 1 and 3 hours; the fourth group – at 0.25, 1.5, 4 and 5 hours after the treatment. The 2-month-old chickens and hens were grouped in groups of 6 animals each. The serum samples (0.5 mL/sample) were obtained from each animal at all intervals.

Experiment II

In the second experiment 93 chickens were included. The drug formulation was administered orally as a 1% water solution at a dose of 30 mg/kg. The solution of the antibiotic was applied intraingluvially using a thin plastic tube. Blood samples were collected prior to drug administration and at 0.17, 0.22, 0.67, 1, 1.5, 2, 3, 4 and 6 post treatment hours. The 7-day-old chickens were grouped in 10 groups of 6 animals each and blood samples were collected from one group at each interval after euthanasia of the animals. The 1-month-old chickens were grouped in 4 groups: group 1 (n=3) – untreated and groups 2, 3 and 4 (6 birds each) – treated. The chickens were sampled similarly to experiment I. From each chicken were obtained samples of 0.5 mL/sample. The 2-month-old chickens and hens were grouped in groups of 6 animals each. The serum samples (0.5 mL/sample) were obtained from each animal at all intervals. The hens were included in the second experiment after 30 days wash out period.

The blood samples were placed in plastic tubes and were allowed to clot. Blood was separated by centrifugation, removed and frozen at -18^0C within 4 hours of collection. Assays were performed within 24 hours of sample collection.

The concentrations of colistin sulfate were assayed microbiologically by the agar diffusion method using *E. coli ATCC 25922* as test microorganism and nutrient meat-peptone agar (NIZPD, Sofia, Bulgaria). The standard solutions of the substance were prepared in serum from untreated birds as a reference. The detection limit of the assay was 0.07 μg/mL. The response of colistin was linear over the range of concentrations between 0.07–5 μg/mL and the mean correlation coefficient (r) of the standard curves was 0.993. Assay validation indicated an intra-assay CV of 2.57 and an inter-assay CV of 8.2.

Pharmacokinetic analysis of serum concentrations after i.v. administration for each bird was performed using a computer program (Topfit V 2.0). The pharmacokinetic parameters were calculated using noncompartmental pharmacokinetic method (Gibaldi, 1984). The calculated pharmacokinetic parameters were: AUC – area under the serum concentration-time curves; MRT – mean residence time, C_{max} – maximum serum levels; T_{max} – time of C_{max}; F_{abs}% – absolute bioavailability. The area under serum concentration-time curve (AUC) was calculated by the method of trapezoids and extrapolation to infinity was made.

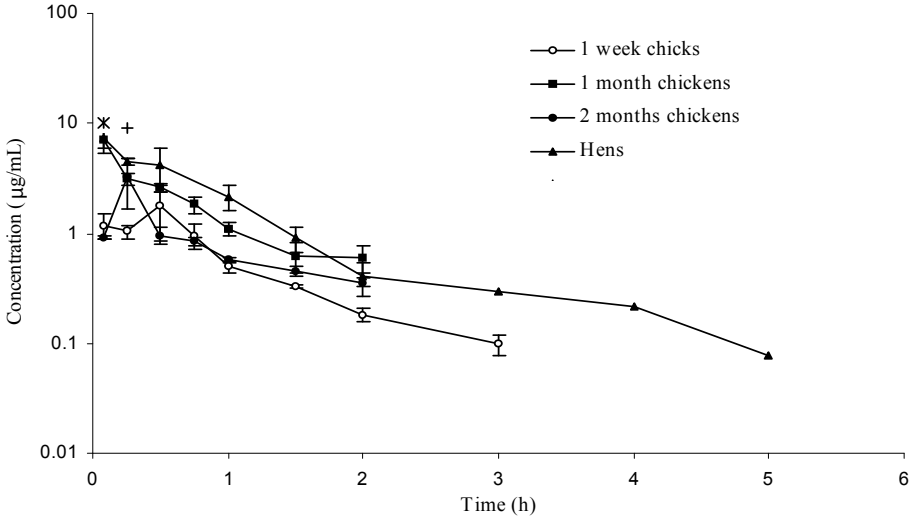

Fig. 1. Serum concentrations of colistin sulfate (mean±SEM), administered intravenously (i.v.) (at dosage of 1 mg/kg) to chickens at different age. * Statistically significant differences at p≤0.05 between 1-month-old and 2-months-old chickens; + Statistically significant differences at p≤0.05 between 1-week old and 1-month old birds and between 1-week-old chicks and hens.

The absolute bioavailability (F_{abs}%) and was calculated according to the equation:

$$F_{abs}, \% = (AUC_{po} \times D_{iv}100)/(AUC_{iv} \times D_{po}).$$

The pharmacokinetic parameters of colistin sulfate were presented as mean ± SD. They were calculated with the *Statistica* computer program (Statistica for Windows, StatSoft Inc. 1993). Statistically significant differences were determined by the Friedman-ANOVA test with Mann–Whitney U–test as a *post hoc* test.

The dose of 1 mg/kg, administered intravenously, provoked depression in the treated birds for a short time. The serum concentrations at 0.083 h were lower than the serum levels found at 0.25 h, probably because of cardiovascular changes. In previous experiments of ours (Lashev and Haritova, 2003) carried out on broiler chickens, a similar effect was observed after the same route of administration. It seems that the toxic reaction depends on the breed, age or other factors. Colistin serum levels were found till the 5th hour after administration in hens and till the 3rd hour in the one–week old chicks. In one 2-months-old chicks, serum levels were detected till the 2nd hour. Therefore, only the AUC values were computed. Its values revealed significant increase in 1–month-old chickens and hens vs 1-week- old chickens and between 2–month-old chickens and hens. Therapeutic concentrations (over MIC of 0.2 μg/mL) were measured up to 2 and 4 hours after injection in the investigated groups (Fig.1).

After p.o. administration serum levels were low and varied within a wide range.

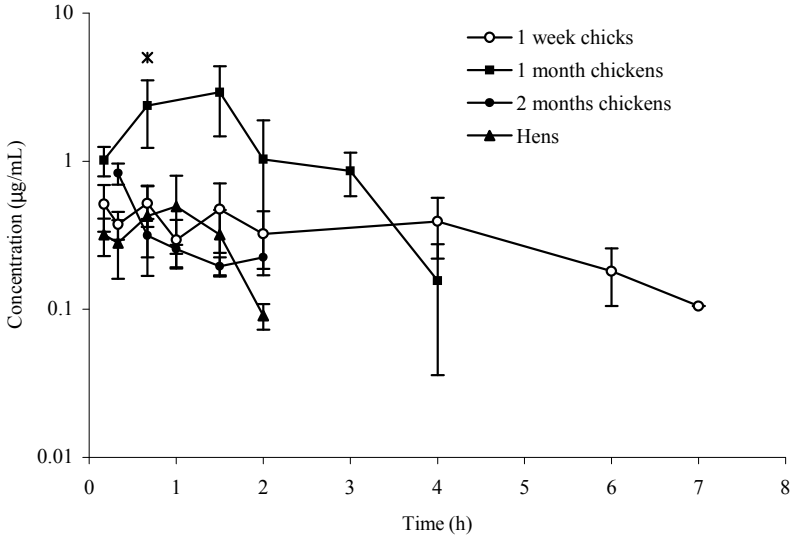

Fig. 2. Serum concentrations of colistin sulfate (mean±SEM), administered orally (p.o.) (at dosage of 30 mg/kg) to chickens at different age; * Statistically significant differences at p≤0.05 between 1-week-old chicks and hens.

Table 1. Pharmacokinetic parameters of colistin after i.v. (1 mg/kg) and p.o. (30 mg/kg) administration in chickens at different age, presented as mean ± SD

Parameters	Age			
	1 week	1 month	2 months	12 months
i.v. administration				
AUC (µg/mL.h)	2.06±0.59	4.31±0.41*	2.62±0.68^	5.11±0.65*
p.o. administration				
C_{max} (µg/mL)	0.70±0.50	2.92±3.24§	0.85±0.19	0.59±0.29
T_{max} (h)	0.56±0.20	1.22±0.28§	0.44±0.11	0.47±0.26
AUC (µg/mL.h)	2.60±0.81	7.16±1.51§	0.59±0.20	2.20±1.40
MRT (h)	2.97±0.27	1.83±0.30§	1.07±0.20	1.41±0.16
F (%)	4.74	5.29§	0.97	0.40

n – number of birds; AUC – area under the concentration-time curves; MRT - mean residence time, C_{max} – maximum serum concentrations, t_{max} – time of C_{max}, F – bioavailability; * statistically significant differences at p≤0.05 towards 1-week-old birds; ^ statistically significant differences at p≤0.05 between 2-months-old birds and hens; § values related to chickens, in which serum concentrations were found (n=3).

These results did not allow to find any subordination related to age. Among 1-month-old chickens serum concentrations were found only in three individuals. Therefore, statistically significant differences were not found between this group and the 1-week-old, 2-month-old chickens and hens. The maximum blood levels were measured in the interval 30 min – 1 h after the treatment and their values were similar in 1-week-old, 2-month-old chickens and hens (Fig. 2).

A statistically significant difference was not found between the computed pharmacokinetic parameters in the four investigated groups (Table 1). A clearer tendency was the 10-times decreasing of the bioavailability of colistin after one month of the age (Table 1). The values of the bioavailability, found in 1-month-old birds were very close to those found previously in broiler chickens – 4.93% (Lashev and Haritova, 2003).

In view of the degree of the differences in the pharmacokinetics between investigated age groups, it could be said that the pharmacokinetics of colistin sulfate was influenced by the age mainly with regards to the persistence of blood levels. Taking into consideration the lack of metabolism, the observed tendencies could be connected with the function of the kidneys as a primary route for colistin excretion, including their maturation.

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