Pharmacokinetic of florfenicol (Water soluble formulation) in healthy and Pasteurella infected broiler chickens

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Abstract: Florfenicol has been approved in the European Union for use in cattle and pigs as an injectable solution for treatment of respiratory diseases in cattle through injection .But now, it was introduced in some countries as an oral solution for the treatment of several poultry diseases. **The aim** of the present study is to describe the Pharmacokinetics of florfenicol (water soluble formulation)in broiler chickens after either a single intravenous and oral administration (by a dose of 30 mg/kg^{-1} body weight). Meanwhile, comparing its disposition in control healthy and *Pasteurella*-infected broilers. Following the IV administration of the drug in healthy and diseased birds, the drug plasma concentration declined in a biphasic pattern. The maximum plasma concentration of florfenicol in control healthy and diseased was reached one hour after its oral administration. But the peak level detected in control broilers was higher than that detected in infected birds. **Conclusion:** Data of the present study showed that volume of distribution, total body clearance in infected birds were higher than that determined in healthy ones. On the other hands, systemic bioavailability were significantly lower (F %,55.6 %) in diseased broiler compared to values determined in healthy ones (F % ,71.5).

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1. Introduction:

Florfenicol, (FF) is a structural analogue of thiamphenicol, possessing a wide spectrum of activity against both Gram-negative and Gram-positive bacteria (Syriopoulou *et al.,* 1981). Florfenicol was reported to have a greater activity than chloramphenicol and especially against *Pasteurella, Salmonella, E. coli* and *Staphylococcus aureus.* Florfenicol inhibits peptidyltransferase activity and affect microbial protein synthesis (Cannon et al., 1990).

The p-nitro group of chloramphenicol is responsible for serious bone marrow toxicity and dose-independent irreversible aplastic anemia, partially described in human, but not in animals. For this reasons, the use of chloramphenicol in food-producing animals has been banned in the USA, the European Union and several other countries.

Florfenicol has been approved in the European Union for use in cattle and pigs as injectable solution for treatment of respiratory diseases in cattle but now it introduced in some countries as oral solution for the treatment of several poultry diseases. The efficacy and residual pattern of water soluble formulation of florfenicol in broilers were described by El-Banna et al., (2007). The disposition kinetic of florfenicol injectable

formulation has been described in healthy and experimentally infected broiler chickens (Afifi and Abo El-Sooud, 1997; Shen et al., 2003) and ducks (El-Banna, 1998). No references to data concerning the disposition kinetics of water soluble formulation in poultry could be obtained.The aim of the present study is to describe the Pharmacokinetic of florfenicol (water soluble formulation) in broiler chickens after a single intravenous and/or oral administration at a dose of 30 mg/kg body weight. Meanwhile, comparing its disposition in *healthy* and *Pasteurella*-infected broilers.

2.Materials and Methods:

2.1. Materials:

2.1.1.Birds

Sixteen symptom-free control healthy broiler chickens and 34 naturally *Pasteurella*-infected (diseased) were used.

-Their body weight ranged from 1.5 to 1.8 kg and their was age 35 days.

-Birds were housed in cages, fed on antibacterial-free balanced rations *ad-libitum* with free access for water.

-Diseased broilers, suffering from slightdiarrhoea, mucoid discharge from the mouth, ruffled feathers, conjuctivits and lack of appetite, were selected from a naturally infected flock.

-Microbiological examination of heart blood samples collected from all used birds revealed that birds were infected with *Pasteurella.* .

-On the other hand, symptom-free broilers were found *Pasteurella*-free.
-Biochemical identification of the isolated strain indicated that the pathogen was *Pasteurella multocida*.
-Analysis of bird plasma revealed no peaks of flofrenicol were seen using the HPLC method of analysis.

2.1.2. Drugs

Florfenicol (Florecol ,100 mg/ml) water soluble formulation for oral use was supplied by Avico (JORDAN). The sterile solutions were prepared by I.V. and oral administration.

2.2.Methods:

2.2.1.Single dose study

A single dose (30 mg /kg-^{1} body weight) of florfenicol was injected intravenously (wing vein) in control healthy and infected broilers (8 birds / group).

Another two groups of 8 control healthy and infected broilers were received florfenicol orally at the same dose (30 mg/ kg-^{1}, b.wt.).

Blood samples (1 ml each) were collected in heparinized tube via wing vein puncture before , after 10, 20, 30 min and 1, 2, 4, 8, 12 and 24 hours post administration.

Blood samples were centrifuged and the clear plasma samples were separated and stored at -20 C until assayed.

***2.2.2.*Multiple doses studies:**

This was performed on the diseased group (18 birds/ group), and given florfenicol (30 mg kg^{-1}, b. wt) daily for 5 consecutive days in drinking water.

Blood samples (1ml each) were collected at 24, 48, 72, 96 and 120 hours from the starting time of giving the dose to estimate florfenicol blood concentrations.

Three birds were slaughtered at 1 hour then at 1, 2, 4, 6 and 7days after the last dose. Blood and tissue samples (lung, liver, kidney, and muscles) were collected for estimation of the drug concentration.

***2.2.3.*Analytical method**

The plasma concentrations of the examined florfenicol were measured by means of a modified reverse-phase high-performance liquid chromatography (HPLC) method reported previously by Varma et al. (1986).

A Shimadzu HPLC system (JAPAN) equipped with auto sampler and detector uv. SPD – 10 AVP detector (Shimadzu) and a Chromolith

Performance RP-180 4.6–100 mm column (Merck KGoA Darmstadt, Germany) were used for the separation and quantification of the drugs. The mobile phase was established on mixture of acetonitrile and water (18:82) at a flow rate of 1 mL/min. The drugs were detected by UV absorption at 224.1 nm.

Plasma or tissue samples were extracted in ethylene acetate (0.5 ml: 1.5 mL or 1g :5ml). The tubes were rotated for 10 min and then centrifuged at 2000 g for 10 min as well. Then, 1 mL of the organic layer was aspirated and evaporated under nitrogen. Each of the residues was dissolved in 0.375 mL of the solvent mixture of acetonitrile–water (1:2, v/v), vortexed, and then centrifuged at 19 000 g for 20 min at 4 ºC. The supernatant was collected, filtered through a 0.45-mm nylon filter, and finally transferred to auto-sampler vials.

Assay validation for Florfenicol indicated a limit of detection (LOD) of 0.01 ug/mL, limit of quantification (LOQ) of 0.05 ug/mL whereas the recovery rates were higher than 92.3% for all florfenicol.

The serum protein-binding of the drug was determined in vitro using the method of Craig and Suh (1980) with florfenicol concentration of 0.625, and 10µg/ml^{-1}

2.2.4.Pharmacokinetic analyses of the data

A computerized curve-stripping program (R Strip; Micromath Scientific Software, Salt Lake City, UT, USA) was used to analyze the concentration-vs-time curves for each individual bird after the administration of florfenicol by both routed. The following intravenous injection, the disposition curve of florfenicol that expresses the decline in drug concentration as a function of time was best described by a bi-exponential expression. The following equation was used to describe the bi-exponential concentration-time curve for florfenicol in serum after intravenous administration:

$$\mathbf{Cp^{\circ} = Ae^{-\alpha t} + Be^{-\beta t}}$$

Cp° is the concentration of drug in the serum at time t.
A is the intercept of the distribution phase with the concentration axis expressed as µg ml^{-1}.
B is the intercept of the elimination phase with the concentration axis expressed as µg ml^{-1}.
α is the distribution rate constant expressed in units of reciprocal time (h^{-1}).
β is the elimination rate constant expressed in units of reciprocal time (h^{-1}), and **e** is the natural logarithm base.
Following administration, each individual curve of florfenicol-vs- time was analyzed to determine the peak of drug concentration (C_{max}) and time to peak concentration (T_{max}). This program also calculated non-compartmental parameters by statistical moment theory. Elimination of half-life ($t_{½el}$) was calculated as Ln 2/β.

The area under the concentration-time curves (AUC) were calculated by the trapezoidal rule, (Gibaldi and Perrier, 1982) and further extrapolated to infinity. AUC is the area under the curve. Systemic bioavailability (F%) is the fraction of the oral dose absorbed and calculated from AUC oral / AUC X100. body clearance area calculated according to Baggot (1978).

***2.2.5.*Statistical analysis**:

The obtained results were presented as mean ± standard error (SE). These results were statistically analyzed using student "t" test, according to **(**Snedecor and Cochran, 1980)

3.Results:

3.1.**The plasma concentration of florfenicolin tested groups**:

Table 1 : Pharmacokinetic parameters of florfenicol in healthy and Pasteurella infected chickens (diseased) after a single intravenous injection of 30 mg /kg b.wt.

Parameter	Unite	Healthy	Diseased
CP^o	µg/ml	76.5 ± 4.3	53 ± 2.35 ***
α	h^{-1}	4.1 ± 0.06	4.4 ± 0.07 **
$t_{½}$ α	H	0.17 ± 0.01	0.16 ± 0.01
β	h^{-1}	0.19 ± 0.001	0.25 ± 0.002 ***
$t_{½}$ (β)	H	3.65 ± 0.11	2.77 ± 0.15 ***
K12	h^{-1}	2.5 ± 0.01	2.7 ± 0.01
K21	h^{-1}	0.85 ± 0.001	1.09 ± 0.02 ***
K el	h^{-1}	0.97 ± 0.02	0.95 ± 0.002
MRT	H	5.3 ± 0.47	4.1 ± 0.11 ***
Vc	L/kg	0.39 ± 0.001	0.57 ± 0.001 ***
Vdss	L/kg	1.3 ± 0.0 2	1.98 ± 0.001 ***
CLB (tot)	L/kg/h.	0.38 ± 0.01	0.55 ± 0. 02 ***
AUC		77.5 ± 2.6	59.3 ± 3.7 ***

(Mean ± S.E., n = 8)

*significant at p ≥ 0.05 ** significant at p ≥ 0.01 *** significant at p ≥ 0.001

The mean plasma concentration of florfenicol in control healthy and infected (diseased) broiler chickens following the I.V.and oral administration of 30 mg kg-1 body weight are recorded in Fig (1 and 2) . Data showed that plasma concentrations of the drug were significantly ($p<0.01$) lower in diseased than in healthy birds at the same time intervals.

Following the I.V. administration of the drug in healthy and diseased birds, the drug plasma concentration declined in a biphasic pattern (Fig1) .

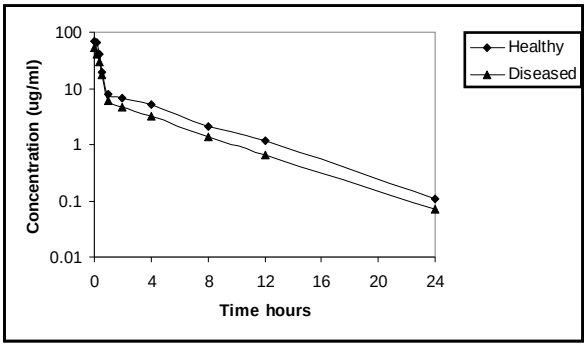

Figure (1): Semilogarithmic graph depicting the time-course of florfenicol in plasma control healthy and diseased broilers after a single intravenous administration of 30 mg kg^{-1}

Following the oral administration of florfenicol with a single dose of (30 mg kg^{-1} b.wt), the maximum plasma level in healthy and in diseased was observed 1 hour post administration (Fig. 2).

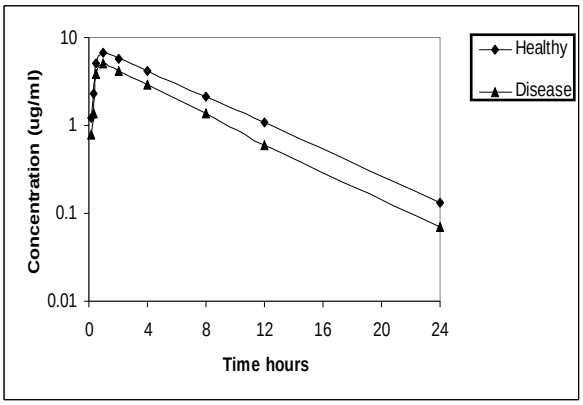

Figure (2) : Semilogarithmic graph depicting the time-course of florfenicol in plasma of control healthy and diseased broilers after a single oral administration of 30 mg kg^{-1}

The drug was detected in concentration of 0.14 and 0.07 ug/ml at 24 hours post oral administration in the healthy and diseased boilers respectively. Pharmacokinetic variables describing the disposition of florfenicol in normal and diseased broilers following intravenous and oral administration were depicted in tables (1 and 2).

Table (2) : Pharmacokinetic parameters of florfenicol in healthy and Pasteurella infected chickens (diseased) after a single after a single oral administration of 30 mg /kg b.wt.

Parameter	Unite	Healthy	Diseased
Kab	h^{-1}	1.9 ± 0.03	2.3 ± 0.02 ***
$t_{½}$ (ab)	h	0.37 ± 0.02	0.30 ± 0.01 ***
MAT	h	3.99 ± 0.21	1.13 ± 0.011 ***
Kel	h^{-1}	0.18 ± 0.001	0.22 ± 0.001 ***
$t_{½}$ (el)	h	3.8 ± 0.01	3.1 ± 0.01 ***
C_{max}	µg/ml	6.8 ± 0.13	5.3 ± 0.2 ***
t_{max}	h	1.4 ± 0.11	1.3 ± 0.1
MRT	h	5.7 ± 0.21	4.3 ± 0.11 ***
AUC	µg.ml.h^{-1}	55.4 ± 2.17	33.2 ± 1.97 ***
F	%	71.5 ± 3.45	55.6 ± 4.27

(Mean ± S.E., n = 8)

3.2.Multiple dose studies

Following the oral administration of florfenicol (30 mg kg^{-1} b.wt) in infected birds daily for 5 successive days, the collected blood samples at 24, 48, 72, 96 and 120 hours showed that Florfenicol was still detected in plasma, and all tested tissues on the 4th day after stopping of drug medication in diseased birds. All tissues of infected birds could be considered drug free except liver and kindeys of infected birds at 6th day after stopping of drug administration (Table 3).

3.3.Protein binding

The capacity of florfenicol binding to plasma proteins was 18.5 and 23.7 % (at 10 µg ml^{-1}) ; and 16.5 and 18.4 % (at 0.625 µg ml^{-1}) with mean values of 17.5 ± 0.82 and 21.05 ± 1.57 % in healthy and diseased plasma; respectively.

Table. (3) : Mean plasma, and tissues concentrations of florfenicol (ug/ml or ug/gm) in pasteurella infected broiler chickens following oral administration of 30 mg/kg b.wt daily for 5 consecutive days.(n = 3).
*** significant at p ≥ 0.001

Tissue	Time of slaughter after the last dose					
	1 h	1st day	2nd day	4th day	6th day	7th day
Plasma	5.2.07 ± 0.09	1.2 ± 0.1	0.6 ± 0.10	0.3 ± 0.02	-	-
Liver	10.4 ± 0.31	4.5 ± 0.11	1.7 ± 0.1	0.5 ± 0.04	0.15 ± 0.01	-
Kidney	9.8 ± 0.45	4.31 ± 0.12	1.6 ± 0.05	0.45 ± 0.03	0.2 ± 0.011	-
Lung	7.1 ± 0.87	3.1 ± 0.23	1.2 ± 0.11	0.32 ± 0.04	-	-
muscle	3.2 ± 0.31	1. 4 ± 0.11	0.9 ± 0.05	0.2 ± 0.03	-	-

- Undetectable.

4. Disscussion

The concentrations of the florfenicol in the plasma were analyzed by means of the same HPLC method.

The results obtained showed lower plasma concentrations of florfenicol in diseased broilers as compared with healthy ones following the drug administration at different time intervals. This observation could be attributed to a more rapid extravascular distribution of florfenicol in diseased than in healthy broilers . The phenomenon of rapid and wide distribution of antimicrobial drugs in diseased tissues have been previously reported in chickens (Soliman, 1989; Atef et al., 1991), and in mammals (Ladefoged, 1979; Baggot 1980).

Our findings showed that plasma concentration of florfenicol injected IV to healthy and diseased broilers follows a two compartment open model. This finding is in agreement with the result previously recorded in broiler chickens (Afifi and Abo El-Sooud, 1997); ducks (Elbanna,1998) and turkeys (Switala,et al.,2007). The reported short distribution and elimination half-lives ($t_{0.5}$ α and β); higher body clearance and the increase in volume of distribution in diseased birds is consistent with the observed lower plasma concentrations of florfenicol in Pasteurella infected broilers. Similar findings have been previously recorded for chloramphenicol in chickens suffering from E. coli infection (Atef et al., 1991). Following IV injection in broilers , florfenicol was rapidly distributed and eliminated. The elimination half-life in healthy broilers ($t_{0.5}$ β) of 3.65 is higher than values recorded in broiler chickens using injectable formulation (2.88 h), Afifi and Abo El-Sooud 1997), turkeys (2.37), Switala,et al.,2007) but shorter than values recorded in ducks (El-Banna, 1998).

In addition, the volume of distribution at steady state (Vdss) and total body clearance (ClB) were also different in different formulation ; being 5.11 L/kg and 26.86 ml/kg/min in broilers (Afifi and Abo El-Sooud 1997) and 1.06 L/kg and 0.32 L/kg/h in turkeys (Switala, et al.,2007) for injectable formulation as compared with 1.3 L/kg and 0.38 L/kg/h respectively for healthy broilers in the present investigation for water soluble formulation.

Following the oral administration, the mean plasma concentration of florfenicol was significantly lower in diseased broilers . This is consistent with the rapid elimination of the drug indicated by the shorter elimination half-life in diseased birds (3.1 h) as compared with the value for healthy ones ($t_{0.5el}$, 3.8 h). Maximum plasma concentrations of florfenicol in healthy and diseased broilers (7.3 and 5.8 µg ml-1) were observed 1 hour post oral administration of the drug. The calculated C_{max} and t_{max} for healthy broilers (6.8 µg ml-1 and 1.4 h respectively) recorded in this study were higher than values recorded previously in broiler chickens (C_{max}, 3.2 µg ml-1 and t_{max} 63.11 + 3.9 min) by Afifi and Abo El-Sooud (1997) but lower than values recorded in turkey (C_{max}, 12.25 µg ml-1 and t_{max} 2 h) by Switala, et al., (2007) . Following the oral administration of florfenicol in a dose of 30 mg kg-1 b.wt. Florfenicol could be detected in plasma of healthy broilers for 19.25 hours following a single IV injection or oral adminsteration in a concentration above the minimum inhibitory concentration (MIC) for pasteurella determined in the present study (0.312 ug/ml) (Syriopoulou *et al.*, 1981).

On the other hand, these levels were obtained for shorter period (15 h) in Pasteurella infected broilers following oral administration.

Until now, studies on the efficacy of florfenicol using pharmacokinetic/pharmacodynamic (PK/PD) approaches have not been carried out. This means that surrogate markers for predicting the clinical effects for florfenicol used in veterinary therapy have not yet been established. On the basis of results obtained in the present studies, we could show graphically that the duration of time that florfenicol concentrations exceed MIC values (T > MIC) characteristic for the susceptible organism were similar. For example, the plasma concentration of florfenicol were maintained above 0.312 ug/mL for 19 and 15 h, respectivelcty in healthy and pateurella infected birds. If one assumes that T > MIC correlates with the efficacy of florfenicol, the differences in the rational dosage regimen based on the PK/PD approach for these drugs would be relative mainly to their pharmacodynamic properties.

For the treatment of infected chickens, a florfenicol oral dose of 30 mg/kg at 12-h interval has been recommended (Afifi & Abo El-Sooud, 1997; Shen et al., 2003). In this study, we have shown that after a single oral dose of 30 mg/kg, the time of florfenicol plasma concentration above 0.31 ug/mL was approximately 15 h in diseased broilers which is in good agreement with Shen et al., (2003). Examination of the pharmacokinetics of florfenicol and its possible adverse effects during continuous administration are necessary for confirming similar dosage in briolers.

Bioavailability value is associated mainly with the degree of bioactivecompound absorption from the gastrointestinal tract and the first-pass effect when the drug particles undergo biodegradation before reaching the central compartment area. Relatively improved florfenicol absorption can be confirmed by its kinetic profile; in particular, florfenicol concentration reaches its maximum value (C_{max},) in the shortest time. This is consistent with shorter absorption live time recorded in the present study. **T**he data obtained showed a relatively lower value of systemic bioavailability F % in diseased broilers (55.6%) as compared with that recorded in healthy ones (F %, 71.5). Similar values was recorded previously in ducks (El-Banna, 1998) but higher values of systemic bioavailability were ,however, previously recorded in broiler chickens (F %, 96.58 %, Afifi and Abo El-Sooud, 1997) following the IM injection and in turkeys following the oral administration (F%, 83 % , Switala, et al., 2007). The capacity of florfenicol binding to plasma proteins was 17.5±0.82 and 21.05±1.57 % respectively in the plasma of healthy and diseased birds. The values in healthy plasma were similar to those reported in broiler chickens (Afifi and Abo El-Sooud, 1997) and in ducks (El-Banna, 1998). The relatively low extent of protein binding of florfenicol was consistent with its high steady-state volume of distribution and extensive distribution in tissues

Our finding revealed that florfenicol concentration in the kidney, and liver was higher than the concurrent plasma concentration. This finding agreed with that reported for florfenicol in poultry (Afifi and Abo El-Sooud, 1997 ;El-Banna, et. al. 2007) and in ducks (El-Banna, et. al., 1998). High drug concentrations in the lung and kidney indicated that florfenicol may be an excellent drug for treating respiratory and urinary tract infections caused by susceptible organisms. The drug was detected in the kidney, and liver of diseased birds until the 6th day after treatment ceased (30 mg kg-1 daily for 5 days).

CONCLUSION

It must be emphasized that it would be unwise to over generalize the findings of this study in relation to all broiler diseases; because clearance rates in birds infected with other different organisms might follow different time courses.

Plasma florfenicol concentrations for 30 mg kg^{-1} daily dosage were suitable to maintain its therapeutic concentration for controlling fowl cholera (Pasteurellosis). In addition, florfenicol should be withdrawn at least 7 days before marketing to ensure that the drug is completely eliminated from tissues.

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