Disposition kinetic and bioavailability of florfenicol in buffalo calves

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The Pharmacokinetics of florfenicol was studied in buffalo calves following single intravenous and intramuscular administration of 20 mg kg^{-1} b.wt. Florfenicol concentration in both serum and urine were determined by microbiological assay using *Bacillus subtilis* (ATCC 6633) as test organism. After intravenous injection the serum florfenicol concentration time course obeys two-compartment open model with distribution ($t_{0.5\ (\alpha)}$) and elimination ($t_{0.5\ (\beta)}$) half lives of 0.381 ± 0.004 and 2.89 ± 0.263 h., respectively. Total body clearance (CL_B) and steady state volume of distribution (Vd_{ss}) were 3.6 ± 0.30 ml kg^{-1} min^{-1} and 1.70 ± 0.010 l kg^{-1}., respectively. After intramuscular administration the observed mean peak serum concentration (C_{max}) was 2.32 ± 0.052 µg ml^{-1} achieved after maximum time (T_{max}) of one hour postinjection. The systemic bioavailability after intramuscular administration was 27.43 % and the plasma protein binding was 13.5 %.

Florfenicol is a new broad-spectrum antibiotic belonging to the thiamphenicol and chloramphenicol. It is a fluorinated derivative of thiamphenicol. A major mechanism of bacterial resistance development to both chloramphenicol and thiamphenicol involves the presence of chloramphenicol acetyl-transferase (CAT) in resistant organisms. The structural modification in the molecule of florfenicol, substitution of a fluorine atom for the hydroxyl group at C3 site, prevents acetylation by CAT (Sams, 1995). Consequently, florfenicol is active against many chloramphenicol resistant strains such as *Klebsiella pneumoniae, Enterobacter cloacae, Shigella dysenteriae, Salmonella typhi, Escherichia coli, Staphylococcus aureus, Proteus vulgaris* and *Haemophilus somnus* (Neu and Fu, 1980; Syriopoulou *et al.,* 1981 and Varma *et al.,* 1986). Florfenicol is recommended for treatment of respiratory infections in cattle (Martel, 1994 and Varma *et al.,* 1991).

The pharmacokinetics of florfenicol have been studied in pigs (Voorspoels *et al.,* 1999), horses (Mckellar and Varma, 1996), cattle (Lobell *et al.,* 1994; De Craene *et al.,* 1997 and Varma *et al.,* 1998), goats (Atef *et al.,* 2000, 2001 and Ali *et al.,* 2003), camel and sheep (Ali *et al.,* 2003), chickens (Afifi and Abo El-Sooud, 1997 and Shen *et al.,* 2002) and ducks (El-Banna, 1998).

The aim of the present study was to determine the pharmacokinetic parameters and bioavailability of florfenicol in buffalo calves in order to establish adequate dose regimen for potential clinical use in buffalo calves infection with susceptible organisms.

Material and Methods

Drug. Florfenicol (Nuflor®, Schering-plough Animal Health, La Grindoliere, France).

Animals. Five healthy buffalo calves weighing 78-84 kg b. wt (6 month old) were used. Animals were kept under good hygienic condition, feed on hay and concentrated mixture and water *ad-libitum.* None of the calves were treated with antibiotics for one month prior to the trial. Experimental design: Animals were given a single intravenous (i. v.) dose of 20 mg kg^{-1} florfenicol into the right jugular vein. Blood samples (10 ml each) were collected from the left jugular vein just before drug administration and at 5, 10, 15, 30-minutes and 1, 2, 4, 6, 8, 12 and 24-hour after drug administration. The blood was allowed to clot at room temperature, then the serum was separated by centrifugation at 3000 rpm for 15 minutes. Each serum sample obtained was divided into two parts, the first was used for determination of florfenicol concentration and the second part was used for creatinine assay. Serum samples were stored at –20ºC until assayed. After a washout period of two weeks, animals injected intramuscularly with the same dose into the deep gluteal muscle of hindquarter and blood was collected and processed as mentioned above.

Urine samples. Each calf was catheterized using folly catheter (No. 14). The bladder was emptied

before drug administration. Urine samples were hours after drug administration for both routes. All urine samples were divided into two parts, the first was used for determination of florfenicol concentration and the second part was used for creatinine assay. Urine samples were stored at -20ºC until used for assessment.

Drug assay. Florfenicol concentrations in serum and urine samples were determined by the microbiological assay method described by (Arret *et al.* 1971) using *Bacillus subtilis* (ATCC 6633) as test organism. Standard curves were constructed using antibacterial-free sera and urine collected from calves. Six wells, 8 mm in diameter were cut at equal distances in standard Petri dishes containing 25 ml seeded agar. The wells were filled with 100 µl of either the test samples or florfenicol standards. The plates were incubated at 37ºC for 16-18 hours. The inhibition zone diameters were measured and the florfenicol concentrations in the test samples were calculated from the standard curve. The lower detectable limit of the florfenicol assay was 0.07 ug ml^{-1}. Semilogarithmic plots of the inhibition zone diameter versus standard florfenicol concentrations in serum were linear with typical correlation coefficient of 0.990 (for the standard curve).

The extent of protein binding of the drug was determined *in vitro* using the method of (Craig and Suh, 1980) with florfenicol concentrations of 40, 25, 20, 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078 ug ml^{-1} in serum according to the following equation:

$$\textbf{Protein binding \%} = \frac{\text{zone of inhibition in buffer-zone of inhibition in serum}}{\text{Zone of inhibition in buffer}} \times 100$$

Creatinine concentrations in serum and urine samples were estimated according to the method previously described (Siest *et al.,* 1985) to determine the creatinine clearance. Florfenicol clearance and creatinine clearance ratio was calculated to determine the pathway of florfenicol elimination through the kidney.

Pharmacokinetic analysis. The pharmaco-kinetic parameters were calculated according to the metod described by (Baggot, 1978). The experimental constants (A, B, α and β) were used to calculate the actual pharmacokinetic rate constants (K_{12}, K_{21} and K_{cl}) which are associated with the mathematical model. The volume of distribution of the central compartment (V_c) was obtained from the equation:

$$\textbf{Vc (mg kg}^{-1}\textbf{)} = \frac{\text{Dose (ug kg}^{-1}\text{)}}{\text{C}^{o}\text{ (ug ml}^{-1}\text{)}}$$

collected prior and at 0.5, 1, 2, 4, 6, 8, 12 and 24

Where. C^o is the drug concentration at the time of i. v. injection (C^o = A + B). While A and B are zero time serum drug concentration intercepts.

Body clearance (CL_B) expressed in ml kg^{-1} min^{-1} was calculated by the equation:

$$CL_B = Kel \times Vc$$

$$\textbf{Bioavailability \% (F)} = \frac{\text{AUC (intramuscular)}}{\text{AUC (intravenous)}} \times 100$$

where AUC is the area under the serum concentration time curves ($AUC = A\,\alpha^{-1} + B\,\beta^{-}$).

Results

The mean serum concentrations time course of florfenicol after i. v. and i. m. administration are depicted in (Fig. 1). Pharmacokinetic parameters are showed in (Table 1). After i. v. administration of 20 mg kg^{-1} b. wt., the florfenicol serum concentration time data obeys two-compartment open model. The distribution and elimination half-lives were 0.381 ± 0.004 and 2.89 ± 0.263 h., respectively. The steady state volume of distribution (Vd_{ss}) was 1.70 ± 0.010 l kg^{-1} and mean residence time was 7.87 ± 0.898 h.

Florfenicol was rapidly absorbed after i. m. administration with absorption half life ($t_{0.5\ (ab)}$) 0.59 ± 0.02 h. Peak serum concentration (C_{max}) was 2.32 ± 0.052 µg ml^{-} achieved after maximum time (T_{max}) of one hour post administration. The drug was slowly eliminated from blood after i. m. than i. v. administration.

Florfenicol was found to be excreted at high concentration in urine of buffalo calves following both i. v. and i. m. routes and extends up to 24 h post administration as shown in (Table 2), also the florfenicol to creatinine clearance was less than one as shown in (Table 3). The systemic bioavailability of florfenicol after i. m. injection was 27.43 % and the extent of plasma protein binding was 13.50 %.

Discussion

In this study, microbiological assay was used to determine florfenicol concentration in serum and urine of buffalo calves. This method did not, however, distinguish between the active metabolites and parent compound. Because the metabolites are microbiologically active, their presence may not necessarily interfere with determination of therapeutic dosage regimen (Sams, 1994).

Florfenicol pharmacokinetics in buffalo calves have been described by a two-compartment open model after the single i. v. dose of 20 mg kg^{-1} b. wt. Our findings are

Table (1): Pharmacokinetic parameters following intravenous and intramuscular administration of 20 mg kg^{-1} b. wt. florfenicol in buffalo calves (n = 5).

Pharmacokinetic parameters after					
i. v			i. m		
Parameter	Unit	Mean ± SE	Parameter	Unit	Mean ± SE
Cp^o	µg ml^{-1}	42.22 ± 0.879	A	µg ml^{-1}	1.47 ± 0.102
A	µg ml^{-1}	23.40 ± 1.364	B	µg ml^{-1}	2.52 ± 0.097
α	h^{-1}	1.82 ± 0.192	K_{ab}	h^{-1}	1.18 ± 0.060
$t_{0.5(\alpha)}$	h.	0.381 ± 0.004	$t_{0.5(ab)}$	h.	0.59 ± 0.02
B	µg ml^{-1}	18.82 ± 0.485	K_{el}	h^{-1}	0.08 ± 0.006
β	h^{-1}	0.24 ± 0.010	$t_{0.5(el)}$	h.	8.66 ± 0.703
$t_{0.5(\beta)}$	h.	2.89 ± 0.263	C_{max}	µg ml^{-1}	2.32 ± 0.052
K_{12}	h^{-1}	0.66 ± 0.061	T_{max}	h.	1.00 ± 0.00
K_{21}	h^{-1}	0.94 ± 0.081	MRT	h.	8.72 ± 0.779
K_{el}	h^{-1}	0.46 ± 0.071	AUC	ug ml^{-1} h^{-1}	25.84± 0.633
$Vd_{(ss)}$	l kg^{-1}	1.70 ± 0.010	F	%	27.43
Vc	l kg^{-1}	0.47 ± 0.040			
AUC	µg ml^{-1} h^{-1}	94.20 ± 1.93			
MRT	h.	7.87 ± 0.889			
CL_B	ml kg^{-1} min^{-1}	3.60 ± 0.026			

**Protein binding 13.5 %.

Table (2): Urine concentration (Mean ± SE) of florfenicol following intravenous and intramuscular administration of 20 mg kg^{-1} b. wt. in buffalo calves (n = 5).

Time (h)	Urine concentration of florfenicol ug ml^{-1} after	
	i.v	i.m
0.5	1043.33 ± 90.99	121.45 ± 4.27
1	617.21 ± 22.41	256.87 ± 13.82
2	330.21 ± 28.95	171.07 ± 21.43
4	279.84 ± 20.92	103.84 ± 14.08
6	147.95 ± 19.53	74.03 ± 4.95
8	105.11 ± 30.75	42.27 ± 2.73
12	16.01 ± 3.24	33.70 ± 0.86
24	6.71 ± 0.613	11.05 ± 0.55

Table (3): Florfenicol / creatinine clearance ratio following intravenous and intramuscular administration of florfenicol at a dose of 20 mg kg^{-1} b.wt. in buffalo calves (n=5).

Time (h)	i. v			i. m		
	Florfenicol clearance ml min^{-1} 10 kg^{-1}	Creatinine clearance ml min^{-1} 10 kg^{-1}	Ratio	Florfenicol clearance ml min^{-1} 10 kg^{-1}	Creatinine clearance ml min^{-1} 10 kg^{-1}	Ratio
0.5	1.53±0.12	33.85±2.50	0.045±0.0002	7.03±0.235	22.55±2.25	0.312±0.016
1	7.17±0.512	26.09±2.31	0.275±0.015	8.69±0.651	20.76±1.88	0.419±0.35
2	14.5±1.23	24.91±2.35	0.582±0.035	20.33±1.052	20.34±2.05	0.999±0.051
4	15.39±1.33	18.97±1.56	0.811±0.057	20.01±0.956	20.28±1.99	0.987±0.06

similar to those reported in calves (Varma *et al.,* 1986; Adams *et al.,* 1987 and De Craene *et al.,* 1997). However, (Bretzlaff *et al.,* 1987; Lobell *et al.,* 1994 and Soback *et al.,* 1995) found that the disappearance of florfenicol from the serum after i. v. dose was described adequately by a tri-exponential terms. The difference between bi- and tri-exponential terms is unlikely to be of clinical importance.

The initial distribution phase was rapid with ($t_{0.5\ (\alpha)}$) of 0.381 h. Similar finding was recorded in calves 0.380 h (De Craene *et al.,* 1997). The mean elimination half-life ($t_{0.5\ (\beta)}$) 2.89 h., which is similar to that reported in other studies: 2.865 h in veal calves (Varma *et al.,* 1986) and also, closely approaches value of 2.77 h in calves (Varma *et al.,* 1991) while, longer than the values that are recorded in goats 1.185 (Ali *et al.,* 2003) and 0.973 h (Atef *et al.,* 2000) and in sheep 1.01 h (Lane *et al.,* 2004). This variation may be due to species difference. The mean body clearance (CL_B) of 3.6 ±0.36 ml kg^{-1} min^{-1}

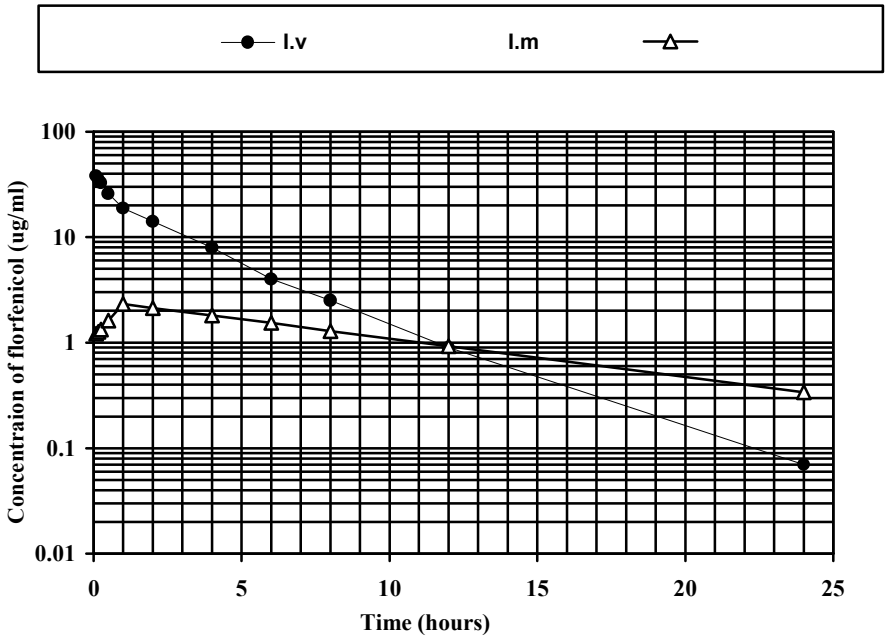

Fig. (1): Semilogarithmic graph depicting the time-concentration of florfenicol in serum of buffalo calves after a single intravenous and intramuscular injection of 20 mg/kg b.wt.

was similar to that is reported in calves 3.57 ml kg^{-1} min^{-1} (Lobell *et al.,* 1994) and 3.67 ml kg^{-1} min^{-1} (De Craene *et al.,* 1997), in goats 4.5 ml kg^{-1} min^{-1} (Ali *et al.,* 2003) and in sheep 6.1 and 4.17 ml kg^{-1} min^{-1} (Lane *et al.,* 2004 and Shen *et al.,* 2004 respectively). The longer $t_{0.5\ (\beta)}$ and smaller CL_B for the drug in buffalo calves compared to those reported in other species are expected. An allometric relationship exists for physiological functions in particular hepatic blood flow, correlated with body weight across different species (Adolph, 1949). By applying principles of allometry to pharmacokinetic parameters (Riviere *et al.,* 1997), the finding of larger clearance for the species with the smaller body weight may be expected. The shorter elimination half-life might be attributed to higher glucuronyl transferase activity in goats (Short *et al.,* 1988).

The volume of distribution at steady state (Vdss) is an accurate indication for the diffusion of the drug in the body tissues (Gilman *et al.,* 1980 and Galinsky and Svensson, 1995). Florfenicol showed Vdss of 1.70 ± 0.010 l kg^{-1}, in buffalo calves, which is similar to its kinetic behaviour in healthy sheep 1.86 and 1.71 l kg^{-1} (Shen *et al.,* 2004). On the other hand, florfenicol showed larger Vdss in broiler chickens (range: 3.50-5.11 l kg^{-1}) as described by (Shen *et al.* 2003 and Afifi and Abo El-Sooud, 1997) and in Muscovy ducks 5.15 l kg^{-1} (El-Banna, 1998). This may be due to individual, anatomical or physiological variations between the different individuals and species.

Florfenicol was rapidly absorbed following the i.m. administration with an absorption half-life ($t_{0.5\ (ab)}$) 0.59 h. This result is in agreement with that is reported in goats (Atef *et al.,* 2000).

The observed mean peak plasma concentration ($C_{max.}$) of florfenicol was 2.32 µg ml^{-1} achieved at ($T_{max.}$) one hour post-injection. Our finding is similar to that is reported in lactating cows 2.3 µg ml^{-1} (Soback *et al.,* 1995), but lower and shorter than the values reported by (Lobell *et al.* 1994) for the florfenicol after i. m. administration to calves and assayed by HPLC (C_{max} 3.21 µg ml^{-1} at 3.33 h). The differences in kinetic parameters are relatively common and are frequently related to interspecies variation, assay method used, extent of blood sampling and

the health status of the animals (Haddad *et al.,* 1985).

Florfenicol showed longer $t_{0.5\ (el)}$ after i. m. administration than i. v. dosing, as it would be slowly released from the site of injection. Intramuscular administration can, therefore, provide an extended period with approximately even concentrations of the drug in the blood.

In this study, the mean level in urine declined after i. v. administration from 1043.33 μg ml^{-1} at 0.5 h to 6.71 μg ml^{-1} after 24 h., while following i. m. administration the drug reached its maximum level 256.87 μg ml^{-1} after one-hour and decreased to its lowest level 11.05 μg ml^{-1} after 24-hour. These values closely approach those in veal calves following i. v. and oral dosing, respectively (Varma *et al.,* 1986). The higher concentrations of florfenicol were found in urine, indicating that florfenicol may be an efficacious drug for treating urinary tract infections caused by susceptible organisms.

The ratios between florfenicol clearance to creatinine clearance was less than one, indicating that the glomerular filtration is the main pathway for florfenicol elimination through the kidney with a variable amount reabsorbed back to blood (Akhtar *et al.,* 1997). The systemic bioavailability (F) of florfenicol in buffalo calves after i. m. injection was 27.43 ± 0.388 %. This value was similar to that is recorded in sheep 27 % (Lane *et al.,* 2004) but higher than that is recorded in cattle 19 % (Sanders *et al.,* 1988) and lower than that in lactating cows 38 % (Soback *et al.,* 1995), calves (range: 59.3 – 106 %) (Lobell *et al.,* 1994) and in goats 60.88 % (Ali *et al.,* 2003) and 65.718 % (Atef *et al.,* 2000). Variability in absorption from the i. m. site might be due to differences in regional blood flow in the different muscle tissue sites which is the major determinant.

In vitro protein binding percentage of florfenicol in serum of buffalo calves was 13.50 ± 0.164 %. This value was similar to its value in calves 13.2 % (Lobell *et al.,* 1994). This finding indicates that the drug is moderately low bound to serum proteins and is consistent with its high steady-state volume of distribution and extensive distribution in tissues.

The minimum inhibitory concentrations (MIC_s) of florfenicol for bacteria isolates from buffalo calves have not yet been determined. Based on MIC data studied on bacteria from calves and cows, the MIC_s of florfenicol for *pasteurella multocida* and *pasteurella haemolytica* ranges from 0.25 to 2.0 μg ml^{-1} with the majority values at 1.0 μg ml^{-1} (Varma *et al.,* 1986), 0.25 μg ml^{-1} (MIC_{90}) is also the level of florfenicol at which 90 % of haemophilus *somnus* inhibited (De Craene *et al.,* 1997). *In vitro*, florfenicol is more active than chloramphenicol against *H. somnus* (Martel, 1994), a major pathogen in bovine meningitis (George, 1996). Florfenicol has a higher therapeutic efficacy in bovine respiratory diseases than other commonly used antibacterials, including amoxicillin, enrofloxacin and oxytetracycline (De Haas *et al.,* 1995; Libersa *et al.,* 1995 and Lockwood *et al.,* 1995).

In this study, the time of plasma concentration above 0.25 μg ml^{-1} is approx-imately 24 h. Therefore, florfenicol should be given 20 mg kg^{-1} b. wt. once daily to maintain therapeutic concentrations in treatment of respiratory infections in buffalo calves. Also, the higher concentrations recovered in urine indicates that the drug would be efficacious in treatment of many Gram-negative urinary tract pathogens.

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