Vet.Med.J.,Giza. Vol.46,No.1.(1998):17-27.

EFFECT OF AFLATOXICOSIS ON THE KINETIC BEHAVIOUR OF CEFTIOFUR IN CHICKENS

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Received : 4/ 11/ 1997
Accepted : 22/ 12/ 1997

SUMMARY

The kinetic behaviour of ceftiofur sodium was studied in aflatoxin treated (for 30 days) and non-treated chickens following oral, intramuscular and intravenous administrations of 10 mg kg^{-1} B. wt. Aflatoxicosis resulted in a significant decrease in ceftiofur serum concentration in the treated rather than non-treated chickens following oral and intravenous administrations. The kinetic behaviour showed that following intravenous injection, the elimination half life time $t_{0.5}$ (el) was significantly shorter in the treated (1.75 ± 0.03 h) than in non treated chickens (4.23 ± 0.05 h). Folowing oral administration, the kinetic behaviour revealed long absorption half-life ($t_{0.5}$ (ab), 62.74 ± 1.59 min) in the treated than non treated chickens (50.46 ± 5.07 min), with low Cmax 23.25 ± 0.42 µg ml-1 at long tmax (3.05 ± 0.07 h) in treated than non-treated chickens (Cmax 27.83 ± 1.28 at tmax 2.39 ± 0.07 h).

INTRODUCTION

Ceftiofur sodium is one of the third generation of cephalosporins antibiotic group. It has a wide spectrum of activity aganist both Gram-positive and Gram-negative bacteria, including some anaerobic bacteria (Brown et al., 1991a). Ceftiofur sodium has been approved for use in several countries for treatment of respiratory diseases in animals (Jaglan et al., 1992).

Several authors studied the pharmacokinetics of ceftiofur in animals, while no references were mentioned concerning its pharmacokinetics in poultry. This work was designed for studying the pharmacokinetic behaviour, tissue distribution and withdrawal time of ceftiofur in normal and aflatoxin treated chickens.

MATERIAL AND METHODS

Aflatoxin:

Aflatoxins, a group of extremely toxic chemicals, produced by certain species of fungi of the genus Aspergillus and can occur as natural contaminants of poultry feed. There are four major aflatoxins B_1, B_2 G_1 and G_2, plus two additional metabolic products M_1 and M_2. Aflatoxin B_1 was produced from Aspergillus flavus species according to the method of Davis et al., (1966).

Cefliofur sodium was obtained as a lyophilized powder (Excenel 4 grams vials) from Upjohn Company, USA.

Chickens

A number of 104 apparently healthy, two weeks old Hubbard broiler chickens were used. The system of light, temperature and humidity were adjusted as rescommended for broiler production. The chickens were classified into two main groups (52 chickens per group).

Experiment I

Effect of aflatoxin B1 on body weight and the activities of AST and ALT.

Chickens of the first main group were fed on balanced diet [Chemical analysis of feed stuffs were applied according to Harrison (1957) and A. O. A. C. (1975). Diet ingredients and composition in Table (1)] free from antibiotic containing 0.75 ppm aflatoxin B_1 Kg_{-1} ration (Pier et al., 1971) for one month before experiment and continued till the end of the experiment. The chickens of the second main group were fed on balanced diet free from both antibiotic and aflatoxin. The chickens of the two main groups were weighed individually at the beginning of the experiment and the average weight was recorded as the initial weight of each group. Body weight in grams was determined after treatment for 30 days. Blood samples were taken from wing vein of the treated and non treated chickens to determine the activity of serum AST and ALT according to Karmen (1955); Reitman and Frakel (1957) and White et al. (1970).

EXPERIMENT II.

Pharmacokinetic of ceftiofur in aflatoxin treated and non-treated chickens:

After the end of experiment I two groups (10birds per group) of aflatoxin treated (aflatoxin was continuously added to the feed) and non-treated chickens, were injected once intravenously with ceftiofur sodium at a dose of 10 mg kg-1 B. wt. Blood samples were collected from wing vein at 15, 30 minutes, 1,2,4,6,8,10 and 12 hours post-injection. For determination of antibiotic concentration in chicken sera. The treated chickens were then left for two weeks to ensure that the drug was completely eliminated from their bodies. The chickens in each group were divided into two subgroups of 5 chickens each. The chickens in each 1st subgroup were given ceftiofur sodium orally (10mgkg-1 b.wt.). The chickens in the two 2nd subgroups were injected intramuscularly with ceftiofur sodium (10mgkg-1 b. wt.). Blood samples were collected from each chicken as mentioned in intravenous injection.

Experiment III.

Repeated administration and tissue distribution of ceftiofur sodium:

Four groups of 21 chickens each (1^{st} and 2nd treated with aflatoxin B1 0.75 ppm for one month.), (3^{rd} and 4th groups were kept as non treated chickens). Chickens of the 1^{st} and 3^{rd} groups were given ceftiofur sodium 10 $mgkg^{-1}$ b.wt orally once for 5 successive days. Chickens of the 2^{nd} and 4^{th} groups were injected intramuscularly with ceftiofur sodium 10mg kg-1b.wt. once daily for 5 successive days. After stop

of drug admnsitration 3 chickens from each group were slaughtered daily for 7 days. Blood and tissue samples (Liver, Kidney, lung, spleen, heart, thigh muscle, breast muscle, gizzard and intestine) were collected for determination of drug concentration.

Assay of samples:

Ceftiofur concentraitons in blood and tissue samples were assayed by microbiological method with *Sarcinea lutea* (ATCC 941a) as a test organism according to Arret et al., (1971).

Estimation of protein binding percent:

Protein binding % of ceftiofur sodium with aflatoxin treated and non-treated chicken sera was estimated according to Lorian (1975).

Pharmacokinetic analysis:

The pharmacokinetic parameters were analysed according to Baggot (1978).

Statisitical analysis:

The obtained results were analysed statistically by "t" test according to Snedecor and Cochran (1980).

RESULTS

Administration of aflatoxin B1 at a rate of 0.75 ppm for 30 days in the fed chickens rsulted in a significant decrease in body weight gain and significant increase in AST and ALT activities (Table 2). The Post-mortem examination of some treated chickens showed lesions of aflatoxicosis (e.g. congested liver and kidneys, fatty liver, splenomegaly, ascites, haemorrhagic enteritis and enlarged proventriculus and gizzard). The alive birds showed anemia, depression, ruffled feathers and anorexia.

Ceftiofur concentrations in the collected sera of chickens fed on aflatoxin containing ration were significantly lower than their concentrations in chickens fed on aflatoxin-free ration following intravenous and oral administrations of ceftiofur at a dose of 10 mg kg-1 b.wt. (Table, 3 and Figs. 1, 2 and 3).

The kinetic parameters of intravenous injection of ceftiofur (10mg kg-1 b. wt.) showed short elimination half-life $t_{0.5(el)}$ in the aflatoxin treated chickens (1.75 ± 0.03 h) than in non treated ones (4.23 ± 0.05 h) Following oral administration, the kinetic parameters revealed a long absorption half-life t0.5(el) (62.74 ± 1.59 min) in aflatoxin treated chickens than in non treated ones t0.5 (ab) (50.46 ± 5.07 min) with low Cmax (23.25 ± 0.42 µg/ml) at long tmax (3.05 ± 0.07 h) in treated chickens than in the non treated ones [Cmax (27.83 ± 1.28 µg ml-1) at tmax (2.39 ± 0.07 h)]. No significant changes in the elimination half-life or interval between doses following oral dosing in the treated and non-treated chickens (Table, 4 and Figs. 1 and 2).

No serum concentrations of ceftiofur could be detected following intramuscular injection in aflatoxin treated and non-treated chickens. Following oral and intramuscular adminsitrations of ceftiofur (10mg kg-1 b. wt.) to the afltoxin treated and non-treated chickens for 5 days no detectable concentrations were recorded in all

Table (1): Diet ingredients and composition.

Ingredients	%	Composition	%
Yellow corn	60	Protein	22.2
Soybean meal	25	Fat	3.4
Egyptian bean	5	Fiber	2.3
Broiler concentrate	10	Soluble carbohydrate	53.5
		Ash	8.4
		Moisture	10.2

Table (2): Body weight, serum AST and serum ALT in chickens fed on normal and aflatoxin-containing ration.

Parameters	Aflatoxin-treated Chickens n = 52	Non afatoxin-treated Chickens n = 52
Body weight (gm)	941.0 ± 36.45**	1449.5 ± 19.90
AST (IU 100^{-1} ml)	204.9 ± 1.21**	174.4 ± 1.34
ALT (IU 100^{-1} ml)	81.7 ± 0.91**	55.3 ± 0.98

** Significant at $P < 0.01$

Table (3) Serum concentration of ceftiofur sodium (ug ml^{-1}) in chickens fed on normal and aflatoxin-containing ration following administration of 10 mg kg^{-1} b.wt. (Mean ± S.E.).

Time	Chickens fed on normal ration.			Chickens fed on aflatoxin containing ration.		
	I/V (n = 10)	Oral (n = 5)	I/M (n = 5)	I//V (n = 10)	Oral (n = 5)	I/M (n = 5)
5 min	73.58 ± 0.22	--	--	33.12 ± 0.8**	--	--
15 min	72.19 ± 0.08	18.13 ± 0.05	--	30.63 ± 0.12**	15.65 ± 0.06**	--
30 min	67.61 ± 0.11	23.00 ± 0.21	--	28.15 ± 0.07**	18.18 ± 0.06**	--
1 h	61.64 ± 0.06	28.19 ± 0.009	--	23.99 ± 0.06**	22.88 ± 0.14**	--
2 h	53.59 ± 0.17	26.26 ± 0.16	--	15.92 ± 0.03**	26.18 ± 0.06**	--
4 h	39.17 ± 0.07	22.60 ± 0.16	--	6.85 ± 0.01**	21.22 ± 0.06**	--
6 h	26.35 ± 0.13	15.90 ± 0.06	--	3.35 ± 0.02**	16.03 ± 0.04**	--
8 h	18.81 ± 0.02	12.20 ± 0.08	--	1.40 ± 0.01**	12.00 ± 0.10**	--
10 h	14.00 ± 0.01	9.60 ± 0.11	--	--	8.80 ± 0.08**	--
12 h	9.95 ± 0.001	7.50 ± 0.05	--	--	6.94 ± 0.02**	--

** Significant at $P < 0.01$. I//V = intravenous I/M = intramuscular

Table (4) Kinetic parameters of ceftiofur sodium following intravenous and oral administration of (10 mg kg^{-1} b.wt.) in chickens fed on normal and aflatoxin containing ration.

Intravenous n = 10				Oral n = 5			
Parameter	Unit	Normal ration	Aflatoxin containing ration	Parameter	Unit	Normal ration	Aflatoxin containing ration
C^o	$\mu g\ ml^{-1}$	73.57 ± 0.16	33.89 ± 0.16**	A	$\mu g\ ml^{-1}$	26.72 ± 2.00	23.07 ± 1.04
K_{el}	h^{-1}	0.1643 ± 0.002	0.3934 ± 0.007**	K_{ab}	h^{-1}	0.9218 ± 0.08	0.6643 ± 0.016**
$t_{0.5el}$	h.	4.23 ± 0.05	1.75 ± 0.03**	$t_{0.5(ab)}$	Min	50.46 ± 5.07	62.74 ± 1.59 **
Vc	$L\ kg^{-1}$	0.1358 ± 0.0002	0.2943 ± 0.0014**	B	$\mu g\ ml^{-1}$	40.18 ± 2.10	34.91 ± 1.59**
$Cl_{(B)}$	$L\ kg^{-1}\ h^{-1}$.	0.0223 ± 0.0002	0.1163 ± 0.0012**	K_{el}	h^{-1}	0.1497 ± 0.007	0.1294 ± 0.0039**
				$t_{0.5(el)}$	h.	5.13 ± 0.62	5.37 ± 0.17
				C_{max}			
				Calc.	$\mu g\ ml^{-1}$	27.83 ± 1.28	23.25 ± 0.42*
				Obs.	$\mu g\ ml^{-1}$	28.19 ±± 0.15	26.18 ± 0.06**
				T_{max}			
				Calc.	h.	2.39 ± 0.15	3.05 ± 0.07**
				Obs.	h.	1.0 ± 0.0	2.0 ± 0.0
				I.b.d.	h.	12.40 ± 0.44	13.75 ± 0.36

* Significant at P < 0.05

** Significant at P < 0.01

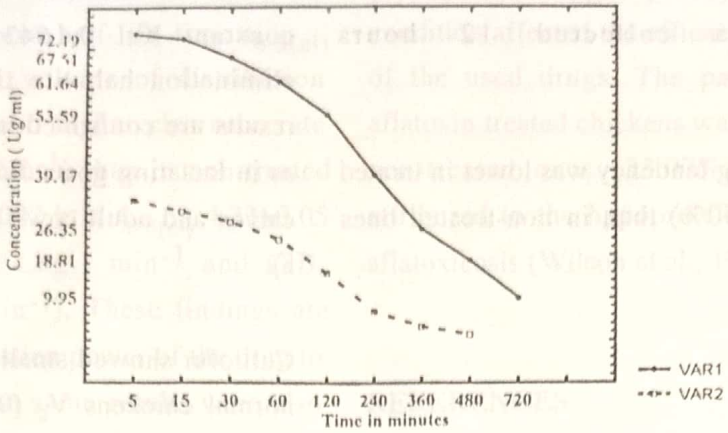

Fig 1 : Semilogarithmic graph depecting the time concentration course of ceftiofur sodium following intravenous injection of 10 mg /kg b. wt. in non-treated and aflatoxin-treated chickens N = 10
VAR 1 = N0n-treated VAR 2 = Aflatoxin-treated

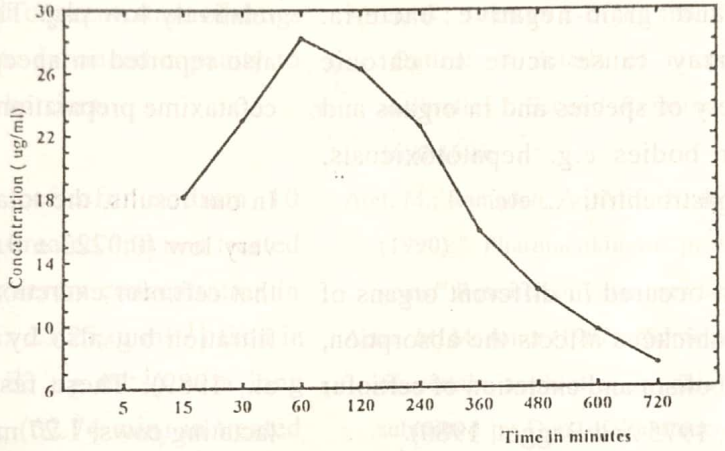

Fig 2 : Semilogarithmic graph depecting the time concentration course of ceftiofur sodium following oral administration of 10 mg/kg b.wt. in non-treated chickens N = 5

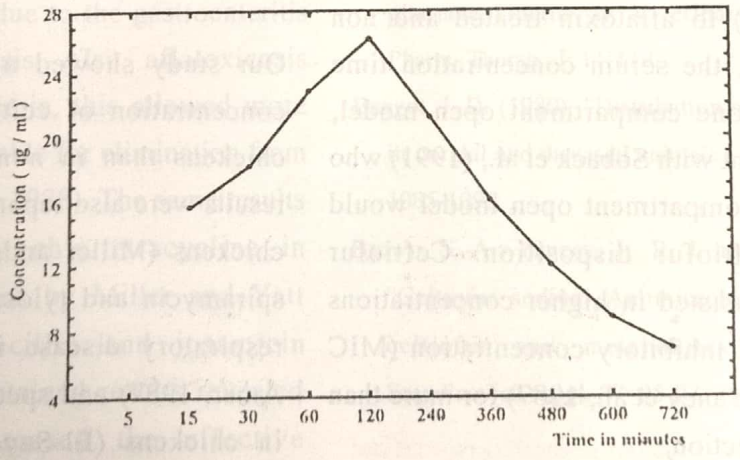

Fig 3 ; Semilogarithmic graph depecting the time concentration course of ceftiofur sodium following oral administration of 10 mg /kg b. wt. in aflatoxin-treated chickens N =5

tissue samples collected 12 hours post-administration.

The protein binding tendency was lower in treated chicken sera (16.50%) than in non-treated ones (23.07%).

DISCUSSION

Ceftiofur sodium is one of the third generation g of cephalosporins of a wide spectrum against Gram-positive and gram-negative bacteria. Mycotoxicosis may cause acute to chronic toxicity in a variety of species and in organs and systems of their bodies e.g. hepatotoxicosis, nephrotoxicosis, gastroentritis ... etc.

The inflammation occured in different organs of aflatoxin treated chickens affects the absorption, distribution, metabolism and excretion of ceftiofur (Pennington et al., 1975 and Baggot, 1980).

Followig intravenous injection of ceftiofur sodium (10 kg-1 b. wt.) to aflatoxin treated and non treated chickens, the serum concentration time curve followed one compartment open model, which is consistent with Soback et al., (1991) who found that one compartment open model would characterize ceftiofur disposition. Ceftiofur concentration persisted in higher concentrations than the minimal inhibitory concentration (MIC 0.06 - 8ug ml-1, Yancy et al., 1987) for more than 10 hours post-injection.

Ceftiofur was highly eliminated from the chicken's bodies, with rapid elimination rate constant Kel 0.1643 ±0.002 h^{-1} and short elimination half-life time $t_{0.5(el)}$ 4.23h. These results are confirmed in different animal species as in lactating goats 3.6 h (Soback, et al., 1989), calves and adult cows 3.5 h (Brown et al., 1991 a).

Ceftiofur showed small volume of distribution in normal chickens V_c (0.1358 ± 0.0002 ml kg^{-1}) indicating poor distribution of the drug to the extravascular tissues. This poor distribution is probably due to its poor lipid solubility and relatively low pk_a. This finding of ceftiofur was also reported in sheep by Atef et al. (1990) on cefataxime preparation.

In our results, the total body clearance $Cl_{(B)}$ was very low (0.0223 ± 0.2 ml kg^{-1} min^{-1}.) indicating that ceftiofur excretion is not only by glomerular filtration but also by extrarenal way (Soback et al., 1989). These results were also reported in lactating cows; 1.27 ml kg-1 min-1 (Soback et al., 1989) and sheep 0.6 ml kg-1 min-1 (Craigmill et al., 1991).

Our study showed a significant lower serum concentration of ceftiofur in aflatoxin-treated chickens than in non treated ones. The same results were also reported for chlortetracycline in chickens (Miller and Yatt, 1985), lincomycin, spiramycin and tylosin in chronic complicated respiratory disease infected chickens (Aziza Amer, 1987) and spectinomycin and spiramycin in chickens (El-Sayed et al., 1994a and b). El-Maaz (1995) for amoxacillin, doxacillin and josamycin. This low serum concentration of ceftiofur in aflatoxin treated chickens is related to

its significant higher elimination rate Kel ($0.3934\pm0.007\ h^{-1}$), short half-life time $t_{0.5(el)}$ (1.57+0.03 h.), higher volume of distribution ($0.293\pm0.001\ L\ kg^{-1}$) and higher clearance rate ($0.1163\pm0.0012 Lkg^{-1}\ min^{-1}$) than in non-treated chickens (Kel, $1543\pm0.002\ h^{-1}$; $t_{0.5(el)}$ 4.23 ± 0.05 h; Vc 0.1358 ±0.0002 Lkg-1 min^{-1} and ClB, $0.0223\pm0.002\ Lkg^{-1}\ min^{-1}$). These findings are due to the higher penetration power of the drug to the diseased tissues. The same results were also reported in infected pigeons (Kosters et al., 1984), in aflatoxin treated chickens (El-Maaz, 1995). Furthermor Welling et al. (1973) and Baggot (1980) attributed the lower concentration of drugs in diseased chickens to its enhanced capacity to penetration of the cellular barriers.

Oral administration of ceftiofur sodium 10 mgkg-1 B. et. to aflatoxin-treated and non treated chickens resulted in lower serum concentration in the treated chickens (Cmax 23.25µg ml^{-1}) than in the non treated ones (27.83 µg ml^{-1}) with long absorption half-life $t_{0.5(ab}$ (62.74 min) in treated chickens than in non treated ones (50.46 min). These findings are due to low absorption of ceftiofur from intestine due to the gastroenteritis produced by aflatoxicosis. Also, aflatoxicosis resulted in hypoproteinemia, this allowed more unbound drug to be available for elimination from the palsma (Wilson et al., 1988). The same results were also reported by chlortetracycline in aflatoxin treated chickens by Miller and Yatt (1984) and by amoxacillin and josamicin (El-Maaz, 1995). Our obtained results revealed that aflatoxicosis decreased the effective concentration of ceftiofur sodium in serum. Which confirmed the findings of Pennington et al. (1975) and Baggot (1980) that this diseased condition affected the efficacy and concentrations of the used drugs. The protein binding % in aflatoxin treated chickens was lower (16.5%) than non-treated ones (23.07%). Which could be attributed to the hypoproteinemia resulted from aflatoxicosis (Wilson et al., 1988)

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