Open Journal of Veterinary Medicine, **2013, 3, 16-21**
http://dx.doi.org/10.4236/ojvm.2013.31004 Published Online March 2013 (http://www.scirp.org/journal/ojvm)

Studies on the Antibacterial Activity of Ceftiofur Sodium *in Vitro* and Birds

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Received December 2, 2012; revised January 4, 2013; accepted February 5, 2013

ABSTRACT

This study was carried out to evaluate the antibacterial activity of ceftiofur sodium as anti-infective chemotherapeutic agent belonged to the third generation of cephalosporins against different bacterial pathogens *in vitro* and birds. The obtained results showed that it was more effective and superior in its action than that the other compared antibacterial agents. The disc diffusion test revealed that most *P. multocida* isolates were highly sensitive to ceftiofur sodium with minimum inhibitory concentrations (MICs) ranging between 0.625 - 2.5 μg/ml and minimal bactericidal concentration (MBCs) equal or two folds of the MICs.

Keywords: Ceftiofur Sodium (Excenel); Microorganisms; Minimum Inhibitory Concentration (MICs); Minimum Bactericidal Concentration (MBCs); Chemotherapeutic; Birds

1. Introduction

Ceftiofur sodium (Excenel) is a newly introduced chemotherapeutic agent for use in veterinary practice [1] not only for large and small animals but also for poultry and fishes against Gram-positive and Gram-negative bacteria [2,3]. The resistance of some bacterial pathogens to existing antimicrobial is wide spread, so continuous research for new drugs for controlling the diseases are necessary. Ceftiofur sodium (Excenel) is one of the third generation cephalosporins. It is a broad spectrum antibiotic active against both Gram-positive and Gram-negative bacteria, including β-lactamase producing strains. It is bactericidal; destroying bacteria by preventing the synthesis of the cell wall [4,5]. It is used for treatment of respiratory tract diseases in cattle, sheep, horse and swine that are caused by *P. multocida* and *P. haemolytica* [6-10]. It was reported for the control of *P. multocida* infection in balady chickens [2], and also for the control of terminal bacterial infection in one day old broiler chickens [11], and American black ducks [12]. The effect of therapeuticceftiofur administration to dairy cattle on *E. coli* was studied [13]. This study was planned as an attempt to evaluate the antibacterial activity of ceftiofur sodium (Excenel) against different bacterial pathogens both *in vitro* and *in vivo* (in birds, in comparison with commonly used antibacterial agents.

2. Meterials and Methods

2.1. Tested Organisms

Forty strains of *E. coli*, 32 strains of *S. aureus*, 5 strains of *Pseudomonas aerugonosa*, 15 isolates of *Salmonella spp*., 12 isolates of *Proteus spp*., 10 isolates of *Pasteurella multocida* besides 3 isolates of *C. ovis* were used to check their succeptibility against different antibacterial drugs.

2.2. Antibacterial Agents Used

Ceftiofur sodium (Excenel), enrofloxacin (Uvetril), flumequine, gentamicin, neomycin, streptomycin and ampicillin. Discs of Whattman filter papers were done and soaked intoexcenel solution in water [14]. Each disc contained 1 μg while discs of uvetril, Flumequine, gentamicin, neomycin, streptomycin and ampicillin were supplied from BioMerieux Co., France. A disc diffusion technique of antibiotics sensitivity testing was done [14-17].

2.3. Determination of Antimicrobial Agents

The antimicrobial agents used for determination of MICs were Ceftiofur sodium (Excenel Upjohn Company USA), Enrofloxacin 10% (Amoun Egypt), Flumequine (Amoun Egypt), gentamicin 10%, Neomycin, Streptomycin and Ampicillin (EL-Naser Company). The tube dilution method for determination of minimal inhibitory concentration (MICs) and minimal bactericidal concentration (MBCs) were done for *P. multocida* (as representative bacterial isolates) according to a reported method [18].

2.4. Experimental Design

Seventy Hubbard chicks of 20 days old with average 200 gm body weight were divided into three groups each of

20 chicks and 10 of them were left as a control. First group received *Salmonella gallinarum* with an intraperitoneal inoculation of infective dose 6×10^4 viable cells [16-19]. Chicks of second group succumbed to an artificial infection with *P. multocida* with an intramuscular dose of 1×10^4 viable cells/ml [10,20], while each chick of third group was intravenously inoculated with 0.1 ml of broth culture of *E. coli* containing 10^8 viable cells/ml [21]. All inoculated and control birds were daily observed and reared under strict hygienic measures. When the characteristic signs of the induced disease appeared, each inoculated bird received an intramuscular injection of ceftiofur sodium (excenel) dissolved in sterile distilled water with a dose of 1 mg/kg of body weight (1 ml of reconstituted sterile solution of excenel per 50 kg. of body weight) as it was recommended by manufacture (Pharmacia and Upjohn, Animal health, Kalamazoo, MI 49001 USA) in a trial to evaluate its action to relieve the symptoms of such avian pathogens.

Two flocks of fattening Hubbard breed chicks each of 5000 birds bred in two floor farms (lower and upper floors, private farm, Sharkia Governorate) were used to make field application of excenel treatment. The birds of first flock received twice application; the first one was daily administration for the first three days of life with a dose 1 mg Excenel/kg B.wt. via drinking water (1 ml of Excenel solution per 50 kg B.wt.); while the second application was done at 30 days of their life with a same dose. The birds of the second flock received no Excenel but their treatment program depended on other antibiotics rather than excenel and served as control. The birds of both flocks received fattening balanced ration contained coxistac as anticoccidial agent for 45 days and were routinely vaccinated against I.B.D. and Newcastle disease. The mortality rate, general health condition and food conversion rate were the parameters of comparison between the birds of both flocks.

Statistical analysis of results were carried out according to a reported method [22].

3. Results

3.1. Results of *in Vitro* Antibiotics Sensitivity Test

It revealed that 39 out of 40 tested strains of *E. coli* were sensitive to ceftiofur sodium with an activity of 97.5%, 30 strains of *S. aureus* were also sensitive with activity percentage of 93.75%, for *Ps. aeruginosa* the activity percentage of 80% was recorded to ceftiofur sodium as 4 strain were sensitive from the five tested isolates, 13 isolates of *Salmonella species* were sensitive to Excenel discs with activity of 86.7%, all tested isolates of *Proteusspecies* were completely sensitive (100%), 9 strains belonged to *P. multocida* were sensitive with activity of 90% and all tested strains of *C. ovis* were completely sensitive to ceftiofur sodium (100%), such superior action of ceftiofur sodium disc was compared with the action of other antibiotic discs *in vitro* on the same tested microorganisms as it was tabulated in **Table 1**.

3.2. Tube Dilution Method for Determination of MICs and MBCs for *P. multocida* Strains

The means zones of inhibition, MICs and MBCs for ceftiofur sodium and other antimicrobial agents against *P. multocida* strains are shown in **Table 2**. Most of *P. multocida* strains showed a high degree of sensitivity to ceftiofur. Fifty percent of tested strains were inhibited by

Table 1. Results of antibiotics sensitivity testing against ceftiofur sodium compared with commonly used antibiotics *in vitro*.

Chemotherapeutic disc disc	Potency	*E. coli* (40)		*S. aureus* (32)		*Ps. aeruginosa* (5)		*Salmonella spp.* (15)		*Proteus spp.* (12)		*P. multocida* (10)		*C. ovis* (3)	
		a	b	a	b	a	B	a	b	a	b	a	b	a	b
Ceftiofur sodium (Excenel)	1 µg	39	97.5	30	93.75	4	80.0	13	86.7	12	100.0	9	90.0	3	100.0
Enrofloxacin	5 µg	37	92.7	28	87.5	3	60.0	12	80.0	11	91.7	8	80.0	2	66.7
Flumequine	30 µg	28	70.0	15	46.88	3	60.0	11	73.3	10	83.3	4	40.0	2	66.7
Gentamicin	10 µg	27	67.5	25	78.13	3	60.0	10	66.7	9	75.0	6	60.0	2	66.7
Neomycin	30 µg	21	27.5	17	53.13	1	20.0	8	53.3	5	41.7	2	20.0	1	33.3
Streptomycin	10 µg	12	30.0	10	31.3	0	00.0	5	33.3	3	25.0	1	10.0	0	00.0
Ampicillin	10 µg	16	40.0	9	28.13	1	20.0	6	40.0	2	16.7	1	10.0	0	00.0
R			R		R		R		R		R		R		R
T-test			T-test		T-test		T-test		T-test		T-test		T-test		T-test

N.B.: a = No. of sensitive strains, b = Percentage of activity.

Table 2. *In vitro* determination of inhibition zone, MICs and MBCs to some representative *P.multocida* strains against different antimicrobial agents.

Antimicrobial agents	Inhibition zone (mm)		Minimal inhibitory concentration (MICs) µg/ml			Minimal bactericidal concentration (MBCs) µg		
	Range	Mean	Range	MIC50	MIC90	Range	MBC50	MBC90
Ceftiofur sodium	18 - 30	24	0.625 - 2.5	0.625	1.25	0.625 - 2.5	1.25	2.5
Enrofloxacin	15 - 28	21.5	0.15 - 1.25	0.31	1.25	0.31 - 2.5	0.625	1.25
Flumequine	10 - 20	15	1.6 - 100	12.5	50	3.1 - 100	25	<100
Gentamicin	12 - 20	16	0.31 - 2.5	0.625	2.5	0.31 - 5	1.5	2.5
Neomycin	10 - 16	13	3.1 - 50	6.25	25	6.2 - 50	12.5	<50
Streptomycin	12 - 26	19	0.625 - 12.5	16	3.1	3.1 - 25	6.3	12.5
Ampicillin	15 - 25	20	20 - 160	40	80	20 - 160	80	<160
R				R	R		R	R
T-test				T-test	T-test		T-test	T-test

0.625 µg/ml and more than 90% of the tested strains were inhibited in a concentration of 1.25 µg/ml. Most of the tested strains were susceptible to enrofloxacin and gentamicin with MICs raning from 0.15 - 1.25 µg/ml and 0.31 - 2.5 µg/ml respectively. Moreover, MBCs was equal or two fold dilutions above MICs for ceftiofur, enrofloxacin and gentamicin. There were a correlation between MICs and inhibition zone on agar. The other antimicrobials had little inhibitory effect against *P. multocida* with MICs 90 values ten to one hundred fold higher compared to ceftiofur.

3.3. Results of Experimental Infection and Treatment

When the characteristic signs of the experimentally induced diseases *i.e. Salmonellosis*, *Pasteurellosis* and *Coli bacillosis* produced, then causative pathogens could be reisolated from such groups of birds. Two days after infection an intramuscular injection of 1 mg/kg B.wt. ceftiofur sodium gave a complete recovery of the inoculated birds of both first and second group while two birds of third group died and the remaining survived.

3.4. Field Application of Ceftiofur Sodium (Excenel) Treatment

The total mortality rate in first flock was 165 with a percentage of 3.3% while it was 7.9% in 2nd flock as 395 birds died, the food conversion rate in first flock was 2.14 while it was 2.26 in 2nd flock as its birds consumed 16.1 tons of food and gave marketd gross weight of 7113 kg as it was tabulated in **Table 3**.

The characteristics of tested organisms were listed in **Table 4**.

4. Discussion

Ceftiofur sodium is registered as Excenel; a trade name imported drug as an antiinfectious agents. The obtained data revealed that *in vitro* testing of antibiotics sensitivity of different pathogens against ceftiofur sodium biodiscs in comparison with other commonly used antibiodiscs, indicated the superiority of the action of ceftiofur sodium biodiscs *in vitro* on the tested microorganisms as the growth of 39 strains of *E. coli* was inhibited with activity of 97.5%, its activity for *S. aureus* was 93.75%, for *Ps. aeruginosa* it was 80%, for *Salmonella spp*. it was 86.7% and 100% for both *Proteus spp*. and *C. ovis* respectively and 90% for *P. multocida*. Such data go in hand with those reported by authors [2,8,23] who stated the efficacy of ceftiofur sodium for the control of *P. multocida* infection in chickens and animals. They were sated that, in disc diffusion test most *P. multocida* strains were highly sensitive to ceftiofur sodium with minimum inhibitory concentration (MICs) ranging between 0.625 - 2.5 µg/ml and minimal bactericidal concentration (MBCs) equal to or double fold MICs. While, MIC90 data for *P. haemolytica*, *P. multocida* and *H. somnus* isolated from bovine pneumonia in the USA and Canada were 0.06 µg/ml with 100% susceptibility (Pharmacia and Upjohn). This difference in MIC for tested microorganisms may be attributed to the difference of the isolated strains from different animals and localites. The difference in susceptibility of tested strains to currently available antimicrobial agents has been documented by some authors [8,19,23-25].

The inhibitory activity of ceftiofur sodium and other antimicrobial against *P. multocida* strains, expressed as minimum and maximum inhibitory concentration, most frequently occurring (model) MIC50, MIC90 (concentration that inhibited at least 90 percent of the tested strains) and inhibition zones are present in **Table 2**. In general, *P. multocida* strains were highly susceptible to ceftiofur, enrofloxacin and gentamicin, MIC90 ranged from 1.25 - 2.5 µg/ml. This study revealed that MBCs for ceftiofur

Table 3. Results of treatment of the experimentally infected groups of birds with ceftiofur sodium (Excenel).

A) Experimental infection				B) Field application		
Character	1st group	2nd group	3rd group	Parameter	1st flock	2nd flock
Infected with:	*S. gallinarum*	*P. multocida*	*E.coli*	Food consumption	17 tons	16.1 tons
Infective dose:	6×10^4	10^{-4}	10^{-9}	Number and percentage of dead birds	165 (3.3%)	395 (7.9%)
Route of infection	I/P	I/M	I/V	Marketed gross weight	9753 kg	7113 kg
Therapeutic	1 mg/kg B.wt.	1 mg/kg B.wt.	1 mg/kg B.wt.	Food conversion rate	2.14	2.26
Results of treatment: a) Drinking water b) I.M. injection	Complete recovery Completerecovery	Complete recovery Complete recovery	2 birds died 18 birds survived Complete recovery	-	-	-

Table 4. The characteristics of tested organisms.

Species	Cultural features	Identification
E. coli	MacConkey agar: aerobic, large 2 - 4 mm, lactose fermenting colonies. Blood agar: 1 - 4 mm colonies, may appear mucoid and some strains are haemolytic.	Gram negative usually motile bacilli.Most strains are indole positive. Indole, methyl red, voges proskauer and citrate utilization IMVC: (++−−).
Staph aureus	Blood and chocolate agar: aerobic, smooth, 1 - 2 mm, golden cream coloured colonies, haemolytic (β haemolysis) due to production of haemolysin. Mannitol salt agar used as a selective medium. Mannitol is fermented giving rise to yellow colonies.	Gram positive cocci, non motile, nonsporing and grape like clusters. Positive coagulase, DNAse and catalase test. Ferment glucose, maltose, lactse, sucrose and mannitol.
Ps. aeruginosa	Blood agar: aerobic, large, flat and haemolytic colonies. Most strains produce pyocyanin and fluorescein pigment (green-yellow in medium). MacConkey agar: non-lactose fermenting colonies with yellow-green in medium. TSI: pink-red slope and butt.	Gram negative motile bacilli. Rapidiy positive oxidase and catalase test. Indole is not produced and liquefy gelatin.
Salmonella species	XLD (xylose lysine deoxycholate) agar: aerobic, pink-red colonies with black centers. Selenite broth is used as enrichment medium. MacConkey agar, DCA, SS agar: colourless non lactose fermenter colonies TSI: pink-red slope and yellow butt.	Gram negative short bacilli. Non sporing and motile except *S. gallinarum* non motile. Indole, V.P., urease and oxidase are negative. Citrate, catalase, H_2S and MR are positive.
Proteus species	MacConkey agar: aerobic, non lactose fermenting pale yellow swarming colonies. Blood agar: produce swarming colonies.	Gram negative bacilli. Non sporing and motile. Urease, gelatin liquefaction and indole are positive. Oxidase test negative.
Pasteurella multocida	Blood agar: aerobic, non haemolytic, Smooth, rough or mucoid colonies. MacConkey agar: no growth as the bile inhibits its growth.	Gram negative coccobacilli with bipolar staining, non sporing, non motile and capsulated. Oxidase (+), ferment sucrose with acid production only, catalase and indole positive. Urease, H_2S and gelatin are negative.
Corynebacterium bovis	Blood agar: aerobic, colonies surrounded by narrow zone of haemolysis. Loeffler's serum give small yellowish white colonies.	Gram positive pleomorphic with palisade and Chinese letter-like arrangement. Ferment glucose and maltose without gas formation. Catalase and urease tests are positive.

was nearly similar to its MICs against most tested strains strongly suggested that ceftiofur exerts bactericidal effect. This results confirmed the findings of most authors [1,13,19,25-27]. They reported that ceftiofur sodium exerts bactericidal effect on tested microorganisms at concentration equal to or at most one doubling dilution above MIC. Many authors were reportedthat high activity of ceftiofur against *P. multocida* isolated from cattle, swine and duck *in vitro* [10,12,28,29]. Also, some authors [8] reported that the MIC of ceftiofur sodium required to inhibit growth of 90% of isolates of *E. coli*, *Pasteurella spp*., *Klebsiella spp*., and beta-haemolytic *Streptococci* was <0.5 microg/ml, and intravenous administration of ceftiofur sodium at rate of 5 mg/kg every 12 h would provide sufficient coverage for the treatment of susceptible bacterial isolates. The present data for ceftiofur sodium confirm this activity. Ceftiofur sodium was superior to many other β-lactamase group with respect to its activity against wide range of Gram-positive and Gram-negative organisms, specially β-lactmase producing strains. Moreover, ceftiofur was converted to dysfuroy1 ceftiofur in serum almost instantly. Dysfuroyl ceftiofur was comparable in potency to ceftiofur against different organisms and *P. multocida* [30]. Mean serum

concentration of ceftiofur and its metabolites peaked approximately one hour after each injection and the highest mean concentration was 5.09 µg/ml. This concentration was five to ten fold above MIC of most tested organisms [6]. The ability of ceftiofur to reduce mortality rate was therefore considerable. The efficacy of ceftiofur was also evident by improved mean body weight gain, feed intake and feed conversion. The improvement of the body gain in response to treatment with ceftiofur is most likely imputable to a proposed improvement of the general health of birds, increase feed intake and increase absorption of nutrients. This previous assumption was supported by some authors [1,19,31]; whose reported that after the lapse of the acute phase of the infection, the drugs improve weight gain in consequence of an increased feed intake and increased absorption of nutrients.

Statistical analysis of results were done and recorded as in **Tables 1** and **2**, which revealed that a significant difference between different parameters ($P > 0.05$).

5. Conclusion

The obtained results revealed that ceftiofur sodium (Excenel) was more effective and superior in its action than the other compared antibacterial agents. Ceftiofur sodium (Excenel) is a chemotherapeutic effective agent for uses in veterinary practice as it action was confirmed both *in vitro* and either in artificially infected or in naturally reared birds.

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