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EXPERIMENTAL AND FIELD TRIALS TO EVALUATE THE ANTIBACTERIAL ACTION OF CEFTIOFUR SODIUM (EXCENEL) IN CHICKENS

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SUMMARY

In vitro and in vivo trials, were done to evaluate the antimicrobial activity of ceftiofur sodium as anti-infective chemotherapeutic agent belonged to the third generation cephalosporins against different bacterial pathogens. The obtained results revealed that it was more effective and superior in its action than the other compared antibacterial agents. In disc diffusion test 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 to or two folds of the MICs.

INTRODUCTION

Ceftiofur sodium (Excenel) is a newly introduced chemotherapeutic agent for use in veterinary practice for not only large and small animals but also for poultry (Abd Allateif and El-Din 1998). The resistance of some bacterial pathogens to existing antimicrobials 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-negative and Gram-positive bacteria, including β-lactamase producing strains. It is bactericidal, destroying bacteria by preventing the synthesis of the cell wall (Yancey et al. 1987). It is used for treatment of respiratory tract diseases in cattle, sheep, horse and swine that are caused by *Pasteurella multocida* and *P. haemolytica* (Brown et al. 1991b, Raemdanek et al. 1994 and Salmon et al. 1996). It is reported for the control of *P. multocida* infection in balady chickens (Abd-Allateif and El-Din 1998) and also for the control

of terminal bacterial infection in one day old broiler chickens (Schriemer et al. 1992). This study was planned as an attempt to evaluate the antibacterial activity of Excenel, as trade name of ceftiofur sodium, against different bacterial pathogens both in vitro and in vivo, in comparison with commonly used antibacterial agents.

MATERIAL AND METHODS

Tested microorganisms:-

Forty strains of *E. coli*, 32 strains of *S. aureus*, 5 strains of *Pseudomonas aeruginosa*, 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 susceptibility against ceftiofur sodium (Excenel), enrofloxacin (Uvetril), flumequine, gentamicin, neomycin, streptomycin and ampicillin. Discs of Whattman filter papers were done and soaked into Excenel solution in water as it was recommended by Bowie and Gould (1952). Each disc contained 1 mg 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 as it was stated by Bauer et al. (1966) and Cruickshank et al. (1975). The activity percentage of each, which is the percentage of the sensitive strains of microorganisms to the totally tested ones was calculated.

The antimicrobial agents used for determin of MICs are: Ceftiofur sodium (Excenel Upj Company USA), Enrofloxacin 10% (Am Egypt), Flumequine (Amoun Egypt), Gentam 10%, Neomycin, Streptomycin and Ampicil (El-Naser Company). The tube dilution meth for determination of minimal inhibitory conce tration (MICs) and minimal bactericidal conce tration (MBCs) were done for *P.multocida* (representative bacterial isolates) according Anon (1991).

Experimental Design:-

a) Seventy Hubbard chicks of 20 days old wi average 200 gm body weight were divided in three groups each of 20 chicks and 10 of the were left as a control. Each chick of 1st grou received *Salmonella gallinarum* with an intra peritoneal inoculation of infective dose 6 x 10 viable cells as it was reported by Ross et a (1955). Chicks of 2 nd group succumbed to a artificial infection with *P. multocida* with a intramuscular dose of $1x10^4$ viable cells/m (Hungerford 1968) while each chick of 3 r group was intravenously inoculated with 0. ml of broth culture of *E.coli* containing 10 viable cells/ml as it was recommended b Gross and Domermuth (1980). All inoculat and control birds were daily observed a reared under strict hygienic measures. Whe the characteristic signs of the induced disea appeared, each inoculated bird received a

intramuscular injection of ceftiofur sodium (Excenel) dissolved in sterile dist. water with a dose of 1mg/kg. of body weight (1ml of reconstituted sterile solution of Excenel per 50 kg. of body weight) as it was recommended by manufacturer (Pharmacia & Upjohn, Animal health, Kalamazoo, MI 49001 USA) in a trial to evaluate its action to relieve the symptoms of such avian pathogens.

b) Two flocks of fattening Hubbard breed chicks each of 5000 birds bred in two floor farms (lower and upper floors, private farm, Kafr Awaad, Sharkia Governorate) were used to make field application of Excenel treatment. The birds of first flock received twice applications; the first one was daily administration for the first three days of life with a dose of 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 day of their life with 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.

RESULTS

Results of antibiotics sensitivity testing in vitro:-

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 strains were sensitive from the tested 5 isolates, 13 isolates of *salmonella species* were sensitive to Excenel discs with activity of 86.7%, all tested isolates of *proteus species* 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).

Results of tube dilution method for determination of MICs and MBCs for *P. multocida* strains:-

The mean 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. 50% of

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

Chemotherapeutic 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	92.5	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

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/ml		
	Range	Mean	Range	MIC_{50}	MIC_{90}	Range	MBC_{50}	MBC_{90}
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

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Table (3): Results of the treatment of the experimentally infected groups of birds and field application of ceftiofur sodium (Excenel).

A. Experimental infection				B. Field application		
Character	**1 st group**	**2 nd group**	**3 rd group**	**Parameter**	**1 st flock**	**2 nd 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 dose :	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	Complete recovery	Complete recovery	2 birds died 18 birds survived	-	-	-
b) I.M. injection	Complete recovery	Complete recovery	Complete recovery	-	-	-

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tested strains were inhibited by 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 ranging from 0.15 - 1.25 µg/ml and 0.31 - 2.5 µg/ml respectively. Moreover, MBCs was equal to 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.

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 2nd groups while two birds of 3rd group died and the remaining survived.

Results of field application of ceftiofur sodium (Excenel) treatment:-

The total mortality rate in 1st flock was 165 with a percentage of 3.3% while it was 7.9% in 2 nd flock as 395 birds died, the food conversion rate in 1 st flock was 2.14 while it was 2.26 in 2 nd one as its birds consumed 16.1 tons of food and gave marketed gross weight weight of 7113 kg. as it was tabulated in table (3).

DISCUSSION

Excenel is a registered trade name of ceftiofur sodium and it is a newly imported drug as an anti-infectious agents. The obtained data revealed that the 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%, it was 100% for both *proteus spp.* and *C.ovis* and 90% for *P. multocida*. Such data go hand in hand with those reported by Scheer (1987) and Abd Allateif and El-Din (1998) who stated the efficacy of ceftiofur sodium (excenel) for the control of *P.multocida* infection in chickens. They were stated that, in disc diffusion test most *P.multocida* strains were highly sensitive to excenel with minimum inhibitory concentrations (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 U.S.A. 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 localities. The difference in susceptibility of tested strains to currently available antimicrobial agents has been documented by some authers (Scheer 1987 and Raemdanek et al. 1992). The inhibitory activity of ceftiofur sodium and other antimicrobial agents 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 persent in Table (2). In general, *P. multocida* strains were higly susceptible to ceftiofur, enrofloxacin and gentamicin, MIC90 ranged from 1.25 - 2.5 µg/ml. This study revealed that MBCs for ceftiofur is nearly similar to its MICs against most tested strains strongly suggested that ceftiofur exerts bactericidal effect. This result confirmed the findings of Franklin (1992) and Klein et al. (1996). 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 have reported high activity of ceftiofur against *P.multocida* isolated from cattle, swine and ducks in vitro (Hariharan et al. 1993; Watts et al. 1993 and Blackall et al. 1996) and the present data for ceftiofur confirm this activity.

Ceftiofur sodium is superior to many other β-lactam group with respect to its activity against wide range of Gram-positive and Gram negative organisms, specially β-lactamase producing strains. Moreover, ceftiofur is converted to dysfuroylceftiofur in serum almost instantly. Dysfuroylceftiofur is comparable in potency to ceftiofur against different organisms and *P. multocida* (Jaglan et al. 1989 and Brown et al. 1991a). 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 is five to ten fold above MIC of most tested organisms (Brown et al. 1991b).

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 the birds, increase feed intake and increase absorption of nutrients. This previous assumption is supported by Alexander (1985) who reported that after the lapse of the acute phase of the infection the drugs improve weight gain in consequance of an increased feed intake and increased absorption of nutrients. With a meticulous vision one may conclude that the newly introduced chemotherapeutic agent, Excenel, is an effective agent for the therapeutic uses in veterinary practice as

action was confirmed both in vitro and either in artificially infected or in naturally reared birds.

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