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In vitro antibacterial activity, postantibiotic effects of norfloxacin and its interaction effects in combination with other antibiotics

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시험관내에서 norfloxacin의 항균력과 다른 항생제와 병용투여시 상호작용

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초 록 : 국내에서 많이 사용되고 있는 제2세대 quinolone 항생제인 norfloxacin(NFX)에 대한 약역학적인 특성을 구명하기 위하여 국내에서 분리된 동물유래 병원성 세균에 대하여 시험관내에서 실험을 수행하여 다음과 같은 결과를 얻었다. 즉, *E coli*(n=89) 대한 NFX의 MIC_{50}과 MIC_{90}은 공히 0.02g/ml이었으며, *Staphylococcus* spp.(n=36)에 대한 NFX의 MIC_{50}은 2g/ml 그리고 MIC_{90}은 4g/ml로 나타났다. *Salmonella* spp.(n=56)에 대한 NFX의 MIC_{50}과 MIC_{90}은 모두 0.2g/ml로 강한 항균력을 보였으며, *Streptococcus* spp.(n=24)에 대한 NFX의 MIC_{50}은 2g/ml 그리고 MIC_{90}이 4g/ml로 나타났다. *Bacillus* spp.(n=34)는 NFX의 MIC_{50}과 MIC_{90}은 모두 0.4g/ml으로서 대부분의 병원성 세균에 대해서 MIC_{50}과 MIC_{90}치가 동일하든지 또는 매우 비슷한 수치를 보여주었다. 그러나 NFX는 혐기성세균인 *Clostridium* spp.(n=34)에 대해서는 항균력이 매우 낮았다. 현재 수의임상에서 항균제 병용요법이 많이 응용되고 있는 것을 고려하여 NFX와 다른 항생물질간의 분획억제농도 (FICs)를 *E coli* 88ac을 시험균주로 하여 실험한 결과, NFX와 colistin과 병용할 때 FIC 값이 0.38로서 상승작용을 그리고 gentamicin, trimethoprim, amikacin, penicillin 및 tylosin과의 병용시 FIC 값이 각각 0.52, 0.56, 0.63, 1.00 및 1.02로서 상가작용을 보여주었으며, tetracycline과의 병용시의 FIC 값은 1.49로서 길항작용을 나타냄을 알 수 있었다. 한편 실제 항균제의 임상적용시 매우 주요한 요소인 항균활성후 저농도유효성(PAE)을 알아보기 위하여 *E coli* AB1157을 시험균주로 측정한 결과 PAE은 0.90~1.02 시간 그리고 *S aureus* R-209에 대해서는 PAE가 1.58~1.99 시간으로서 그람음성균 및 그람양성균 모두에 대해서 긴 PAE를 갖고 있음을 알 수 있었다.

Key words : postantibiotic effect, norfloxacin, combination, MIC, FIC.

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Introduction

Norfloxacin(NFX) is a fluoroquinolone which inhibits the activity of bacterial DNA gyrase, an enzyme which controls the supercoiling of DNA by converting relaxed covalently closed circular DNA to a superhelical form by an enzyme-dependent strand breakage and resealing process. In view of antibacterial spectrum, NFX has an excellent activity against most gram-negative aerobic bacteria[1]. Therefore, the antibacterial agent has been widely used for the treatment of gram-negative severe infectious diseases in animals[2].

Combinations of antimicrobial agents have been frequently advocated by clinicians due to the emergence of resistant pathogenic organisms against single antibacterials. Combinations of antimicrobial agents have been advocated by clinicians due to various reasons: to decrease dose-related toxicity[3,4], to prevent the emergence of resistance[5~7] and/or to enlarge the antimicrobial spectrum[8~10].

Set-up of optimal dosage regimens for antimicrobial therapy is dependent upon pharmacokinetic and pharmacodynamic profiles, in addition to the information on the side effect[11]. Postantibiotic effect(PAE) is another important factor for selecting optimal antimicrobials, especially for bactericides such as quinolones. A long PAE provides the antimicrobials with longer administration intervals[12~14]. With emphasis on the above mentioned aspects, we investigated the *in vitro* activity of NFX against the isolates from the domestic animal origin, together with its PAE. Combination effects of NFX with other important antibiotics were also studied.

Materials and Methods

Antimicrobial agents : All antimicrobials used in this experiment were purchased from commercial sources: penicillin (Sigma), trimethoprim(Dongwha), tylosin(Sigma), colistin (Sigma), tetracycline(Pfizer), amikacin(Sigma), gentamicin (Sigma), and norfloxacin(Sigma).

Test organisms : Test organisms used for determinations of MICs were received from Daesung Laboratory or Veterinary Research Institute during two years from 1993 to 1994. The organisms were isolated from feces of pigs, cattle and chickens with diarrheal symptomes. The isolated bacteria were firstly screened on the basis of colony characteristics by using each selective media(*E coli* and *Salmonella* spp. to DHA medium, *Staphylococcus* spp., *Streptococcus* spp. and *Bacillus* spp. to PEES medium). *Clostridium* spp. was selected by BL medium under ananerobic coditions. Thses bacteria were further confirmed by Mitsuoka method[15]. However, we did not determined in detail species of bacteria.

Minimal inhibition concentrations(MICs) : In order to obtain MICs, agar dilution and broth microdilution methods were performed as outlined by National Committee for Clinical Laboratory Standard Methods[16]. In short, Mueller-Hinton broth(MHB) was adjusted to contain 20mg of calcium and 10mg of magnesium per liter. All media were supplemented with lysed horse blood(2 to 3%). For testing anaerobic bacteria, broth microdilution trays were incubated in anaerobic steel jar. The inocula were adjusted to give approximately $5x10^5$ CFU/ml($1x10^6$ CFU/ml for anaerobes), and MICs were recorded after 16 to 18 h of incubation(48h for anaerobes). Agar dilution tests were performed with inocula of $5x10^4$ CFU/ml.

Fractional inhibition concentrations(FICs) : For the experiment, *E coli* K88ac, *S typhimurium* 1926 and *S aureus* R-209 were kindly provided from Veterinary Research Institute. Combinations of antibacterials were studied by the microdilution checkerboard technique[17]. Fractional inhibitory concentrations(FICs) of each antibiotic, alone or in combination, were noted; FIC indexes were calculated according to the method of King *et al*[18]. Synergism was defined as a FIC index of 0.5 or less. Antagonism was regarded as a FIC index of 1.0 or more. On the other hand, there are addition phenomena which correspond to FIC index between 0.5 and 1.0, excluding both range end numbers.

Determination of PAE : Pre-exposure MICs were determined against *S aureus* R-209 and *E coli* AB1186 by the broth microdilution method according to the guidelines of the National Committee for Clinical Laboratory Standards[16]. The MIC was defined as the lowest concentration of antibiotic that prevented visible growth following an 18h incubation at 37℃. In order to determine the PAE, bacterial

inocula were prepared with three to five colonies picked from a precultured agar plate in MHB and incubated for 3h at 37℃ to bring the organisms into a log-linear growth phase. The starting inoculum for drug-bacterium combination was adjusted to give $1x10^6$ to $1x10^7$CFU/ml in a total volume of 10ml of MHB. Bacteria were incubated in tubes with(2 x MICs) or without drug(control) for 2h. Following the 2h incubation, the tubes were centrifuged at 1, 200 x g for 15min, and 9ml of supernatant was discarded. The remained precipitant(1ml) containg bacteria was resuspended with 9ml of prewarmed fresh drug-free MHB and gently vortexed. This procedure was repeated three times. The number of bacteria was counted by removing 200 l from the culture at wash-out time(2h) and thereafter with hourly intervals up to 8h(*S aureus* R-209) and 9h(*E coli* AB 1157). Samples were serially diluted with cold 0.9% sodium chloride, and 100μl was plated onto MHA in triplicate. The plates were incubated for 18 to 24h, and the colonies were counted. When bacterial counts were expected to be low, 100μl samples were placed in 10ml of cold 0.9% sodium chloride and were drawn through a 0.45μm pore-size filter (Millipore) under suction. Filters were placed aseptically on MHA and incubated for 18 to 24h, and the colonies were counted. The lower limit of detection of the number of bacteria for this procedure was 10 CFU/ml. The PAE was calculated from plots of CFU per milliliter versus time by PAE = T-C, where T is time required for the count of CFU in the test culture to increase 1 x $\log_{10}$(10-fold) above the count observed immediately after drug removal and C is the time required for the count of CFU in an untreated control culture to increase by 1 x $\log_{10}$(10-fold) above the count observed immediately after completion of the same procedure used on the test culture for drug removal[18].

Results

In vitro activity : MIC_{50} and MIC_{90} values of 8 antimicrobial agents for *E coli, Staphylococcus* spp., *Salmonella* spp., *Streptococcus* spp., *Bacillus* sp, and *Clostridium* spp. strains are presented in Table 1. Of the *Escherchia coli*(89) tested, 90% were inhibited by 0.02μg of NFX per ml. The highest MIC_{90} of NFX was 4μg/ml for gram-positive organisms, *Streptococcus* spp. and *Staphylococcus* spp. The MICs for 50%(MIC_{50}) and 90%(MIC_{90}) of NFX against *Salmonella* spp. were 0.2 to 0.2μg/ml, respectively. When NFX was compared with other antibacterials, NFX had a greater activity than those of the other antibacterials: the MIC_{90}s for the *Enterobacteriae* were 0.02μg of amikacin per ml; 0.02μg of colistin per ml; and 8 or 4μg of penicillin per ml. By comparison antibacterial activity of NFX was less active than that of penicillin against *Staphylococcus* spp. and had similar activity to those of penicillin and tetracycline. NFX was the most active compound of antibacterials against *E coli* with 0.02μg/ml(MIC_{90}) and *Salmonella* spp., 0.2μg/ml(MIC_{90}). *Clostridium* spp. were inhibited by penicillin(MIC_{90}: 4μg/ml), amikacin(MIC_{90}: 0.4μg/ml) and tetracycline(MIC_{90}: 8μg/ml). However NFX, tylosin and gentamicin were not active against the organisms.

Combination test : Single antibacterial therapy showed very limited effects in the field situation. Therefore we studied the combination effects between NFX and other antibacterials. Table 2 and Table 3 summarize the MICs of various antibacterials against field-isolated *E coli* K88ac and *S typhimurium* 1926, respectively. The MICs for these strains were each determined three times in duplicate and showed little variation. In the case of the combined effects of NFX and colistin against *E coli* K88ac, fractional inhibitory concentration(FIC) of NFX was 0.08μg/ml which reduced from 0.63μg/ml when used alone, with that of colistin being 0.78μg/ml from 3.13μg/ml. Other highly decreases in MIC were showed in combinations of NFX-amikacin or -gentamicin. Combination of NFX and amikacin reduced 0.63μg/ml and 3.13μg/ml being used alone to 0.08μg/ml and 1.56μg/ml, respectively. The FIC indexes for combination of NFX with colistin, gentamicin, trimethoprim, amikacin, penicillin, tylosin and tetracycline against *E coli* K88ac were 0.38, 0.52, 0.56, 0.63, 1.00, 1.02 and 1.49, respectively(Table 2). The FIC indexes for combinations of NFX with the same antibacterials against *S typhimurium* 1926 were shown in Table 3.

We further investigated their interactions by combination ratios between two antibacterials against *E coli* K88ac and *S*

Table 1. *In vitro* activities of various antimicrobials against 247 bacterial isolates from domestic animals

Microorganis(No. tested)	Antimicrobial agents	MIC(μg/ml) 50%	90%
Escherchia coli(89)	Norfloxacin	0.02	0.02
	Penicillin	4.00	8.00
	Amikacin	0.02	0.02
	Tetracycline	0.50	2.00
	Colistin	0.02	0.02
	Cephazolin	2.00	4.00
	Tylosin	128.00	128.00
	Gentamicin	2.00	4.00
Staphylococcus sp.(36)	Norfloxacin	2.00	4.00
	Penicillin	32.00	64.00
	Amikacin	2.00	8.00
	Tetracycline	0.50	1.00
	Colistin	64.00	64.00
	Cephazolin	0.50	0.50
	Tylosin	16.00	32.00
	Gentamicin	8.00	16.00
Salmonella sp.(56)	Norfloxacin	0.20	0.20
	Penicillin	128.00	128.00
	Amikacin	0.20	0.20
	Tetracycline	0.04	1.00
	Colistin	0.02	0.02
	Cephazolin	2.00	4.00
	Tylosin	128.00	128.00
	Gentamicin	2.00	2.00
Streptococcus sp.(24)	Norfloxacin	2.00	4.00
	Penicillin	0.20	4.00
	Amikacin	4.00	8.00
	Tetracycline	2.00	4.00
	Colistin	2.00	4.00
	Cephazolin	0.20	0.20
	Tylosin	4.00	8.00
	Gentamicin	4.00	16.00
Bacillus sp.(34)	Norfloxacin	0.40	0.40
	Penicillin	4.00	4.00
	Amikacin	0.20	0.40
	Tetracycline	4.00	8.00
	Colistin	2.00	4.00
	Cephazolin	0.50	1.00
	Tylosin	16.00	32.00
	Gentamicin	8.00	16.00
Clostridium sp.(34)	Norfloxacin	128.00	128.00
	Penicillin	4.00	4.00
	Amikacin	0.20	0.40
	Tetracycline	4.00	8.00
	Colistin	2.00	4.00
	Cephazolin	32.00	64.00
	Tylosin	128.00	128.00
	Gentamicin	128.00	128.00

Table 2. Interaction between norfloxacin and various antibiotics against *E coli* K88ac (Unit:μg/ml)

A	B	Single MIC_A	Single MIC_B	Combination MIC_A	Combination MIC_B	FIC index	Interpretation
NFX	PC	0.63	50.00	0.63	0.05	1.00	addition/antagonism
NFX	COL	0.63	3.13	0.08	0.78	0.38	synergism
NFX	TMP	0.63	25.00	0.16	12.50	0.56	addition
NFX	AMK	0.63	3.13	0.08	1.56	0.63	addition
NFX	GM	0.63	3.13	0.04	1.56	0.52	addition
NFX	TYL	0.63	25.00	0.16	25.00	1.02	addition/antagonism
NFX	TC	0.63	0.78	0.31	0.78	1.50	antagonism

FIC index: $(MIC_{A,combination}/MIC_A)+(MIC_{B,combination}/MIC_B)$
NFX, norfloxacin; PC, penicillin; Col, colistin; TMP, trimethoprim: AMK, amikacin; GM, gentamicin; TYL, tylosin; TC, tetracycline.

Table 3. Interaction between norfloxacin and various antibiotics against *S typhimurium* 1926 (Unit:μg/ml)

A	B	Single MIC_A	Single MIC_B	Combination MIC_A	Combination MIC_B	FIC index	Interpretation
NFX	PC	0.63	6.25	0.63	6.25	2.00	antagonism
NFX	COL	0.63	6.25	0.08	0.78	0.25	synergism
NFX	TMP	0.63	25.00	0.31	12.50	0.62	addition
NFX	AMK	0.63	12.50	0.16	6.25	0.63	addition
NFX	GM	0.63	3.13	0.08	3.13	1.03	addition/ antagonism
NFX	TYL	0.63	25.00	0.04	25.00	1.03	addition/ antagonism
NFX	TC	0.63	3.13	1.25	3.13	1.50	addition/ antagonism

FIC index: $(MIC_{A,combination}/MICA)+(MIC_{B,combination}/MIC_B)$
NFX, norfloxacin; PC, penicillin; Col, colistin; TMP, trimethoprim: AMK, amikacin; GM, gentamicin; TYL, tylosin; TC, tetracycline.

aureus R-209. Antibacterial activity according to ratios between NFX and tylosin or colistin evaluated against *E coli* K88ac using checkerboard method. NFX-colistin combination showed a synergism against *E coli* K88ac and *S aureus* R-209(Table 4). Synergistic activity appeared when NFX was in the range of 0.06 to 1.03 parts to colistin as the unity. As the parts of NFX were increased more than 4 to colistin as the unity, the interactions were changed to addition or antagonism. On the while, the combination ratios between NFX and tylosin was studied against *E coli* K88ac in Table 5, which shows some additions. Combination ratio of NFX-colistin(1:1) showed a synergistic effect against *S aureus* R-209(Table 6). Combination of NFX-tylosin against

Table 4. Combinations of Norfloxacin-colistin and their FIC indexes against *E coli* K88ac (Unit:μg/ml)

Alone		Combination		FIC Index	Ratio	
MIC (colistin)	MIC (norfloxacin)	MIC (colistin)	MIC (norfloxacin)		colistin	Norfloxacin
0.63	0.31	0.31	0.02	0.56	1 :	0.06
0.63	0.31	0.31	0.04	0.62	1 :	0.13
0.63	0.31	0.31	0.08	0.75	1 :	0.26
0.63	0.31	0.15	0.16	0.76	1 :	1.03
0.63	0.31	0.07	0.31	1.12	1 :	4.01
0.63	0.31	0.03	0.31	1.06	1 :	8.01
0.63	0.31	0.01	0.31	1.03	1 :	16.45
0.63	0.31	0.01	0.31	1.02	1 :	32.22

FIC index: $(MIC_{A,combination}/MIC_A)+(MIC_{B,combination}/MIC_B)$.

Table 5. Combinations of Norfloxacin-tylosin and their FIC indexes against *E coli* K88ac (Unit:μg/ml)

Alone		Combination		FIC Index	Ratio	
MIC (tylosin)	MIC (norfloxacin)	MIC (tylosin)	MIC (norfloxacin)		norfloxacin	tylosin
12.5	0.78	3.13	0.78	1.25	1 :	4.01
12.5	0.78	1.56	0.78	1.13	1 :	2.00
12.5	0.78	0.78	0.78	1.06	1 :	1.00
12.5	0.78	0.39	0.78	1.03	1 :	0.50
12.5	0.78	0.20	0.78	1.02	1 :	0.25
12.5	0.78	0.10	0.78	1.01	1 :	0.13
12.5	0.78	0.05	0.78	1.00	1 :	0.06
12.5	0.78	0.02	0.78	1.00	1 :	0.03
12.5	0.78	0.01	0.78	1.00	1 :	0.02

FIC index: $(MIC_{A,combination}/MIC_A)+(MIC_{B,combination}/MIC_B)$.

Table 6. Combinations of Norfloxacin-colistin and their FIC indexes against *S aureus* R-209 (Unit:μg/ml)

Alone		Combination		FIC Index	Ratio	
MIC (colistin)	MIC (norfloxacin)	MIC (colistin)	MIC (norfloxacin)		colistin	Norfloxacin
12.5	25.00	12.50	0.39	1.02	1 :	0.03
12.5	25.00	12.50	0.78	1.03	1 :	0.06
12.5	25.00	12.50	1.56	1.06	1 :	0.12
12.5	25.00	12.50	3.13	1.13	1 :	0.25
12.5	25.00	12.50	6.25	1.25	1 :	0.50
12.5	25.00	6.25	12.50	1.00	1 :	2.00
12.5	25.00	1.56	1.56	0.19	1 :	1.00

S aureus R-209 showed similar tendency like that against *E coli* K88ac: addition and antagonism(Table 7).

Postantibiotic effect : In order to evaluate PAE of NFX,

Table 7. Combinations of Norfloxacin-tylosin and their FIC indexes against *S aureus* R-209 (Unit:μg/ml)

Alone		Combination		FIC Index	Ratio	
MIC (tylosin)	MIC (norfloxacin)	MIC (tylosin)	MIC (norfloxacin)		norfloxacin	tylosin
1.56	25.00	1.56	0.39	1.02	1 :	0.25
1.56	25.00	1.56	0.78	1.03	1 :	0.50
1.56	25.00	1.56	1.56	1.06	1 :	1.00
1.56	25.00	1.56	3.13	1.13	1 :	2.00
1.56	25.00	1.56	6.25	1.25	1 :	4.01
1.56	25.00	1.56	12.50	1.50	1 :	8.01
1.56	25.00	1.56	12.50	1.50	1 :	16.03
1.56	25.00	0.78	12.50	1.00	1 :	32.05
1.56	25.00	0.39	25.00	1.25	1 :	64.10

FIC index: $(MIC_{A,combination}/MIC_A)+(MIC_{B,combination}/MIC_B)$.

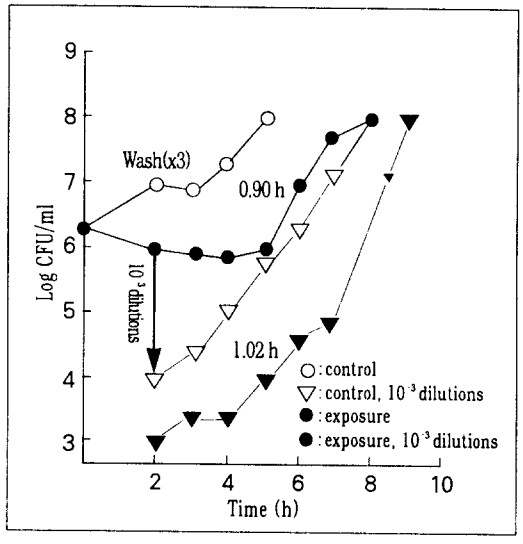

Fig 1. Postantibiotic effects by 2 hours exposure of *E coli* AB 1157 in broth 1.2μg/ml of norfloxacin using rapid drug removal by repeated washing. a 10^{-3} dilutions.

we exposed *S aureus* R-209 and *E coli* AB1157 to the concentrations of two times of MIC and processed as suggested in the Materials and Methods. As shown in the Fig 1. and Fig 2, it was more correctly calculated according to the formula of PAE=T-C. PAE of NFX against *E coli* AB1157 was 1.02 hours and that against *S aureus* R-209 was 1.58 hours under 1,000 times dilution. On the other hand, PAE of NFX against *E coli* AB1157 was 0.90 hours and that against *S aureus* R209 was 1.99 hours when using un-

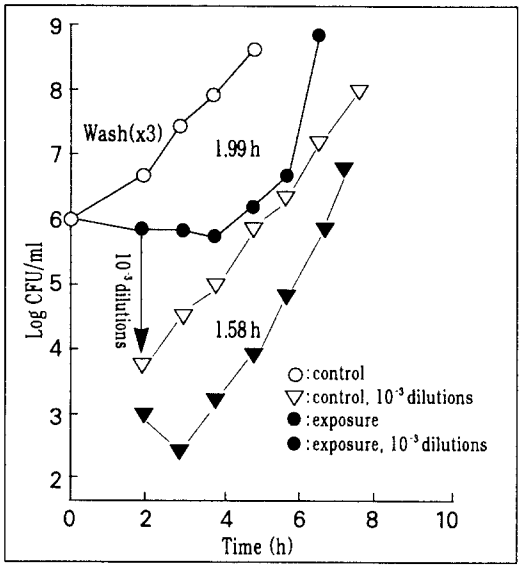

Fig 2. Postantibiotic effects by 2 hours exposure of *S aureus* R-209 in broth 1.2μg/ml of norfloxacin using rapid removal by repeated washing. a 10^{-3} dilutions.

diluted pathogens. Two methods(diluted vs. undiluted) showed almost the same PAE.

Discussion

NFX is a recently developed quinolone that has potent antibacterial activity[1]. The antimicrobials showed activity against the majority of the organisms tested in this study exept *Clostridium* spp.(Table 1). Against enterobacteriae tested, NFX showed good activity, inhibiting 90% of the strains at a concentration of 0.5μg/ml. But MICs of *Staphylococcus* spp., *Streptococcus* spp. and *Bacillus* spp. were above 1μg/ml. Comparatively NFX was slightly less active than penicillin against gram positive bacteria, but its activity was greater than that of tylosin. In the combinations of NFX with other antibacterials, NFX showed the synergism when combined with penicillin, amikacin and colistin. Generally speaking, bactericidals showed the synergistic effects when combining with another bactericides[4,8]. These phenomena were also confirmed in our experiment. As expected, the combination of NFX with tylosin or tetracycline showed the only addition at best or the antagonism, depending on the combination ratios. The most appropriate combination ratios between NFX and colistin against the test organisms were suggested in this experiment. With using these ratios and some background pharmacokinetic data obtained from the target animals, new formulations and dosage regimens could be contributed. The MICs of NFX against *E coli* and *S typhimurium* were between 0.31 and 2.5μg/ml. The MIC_{50} of penicillin, colistin, trimethoprim, amikacin, gentamicin, tylosin and tetracycline against *E coli* and *S typhimurium* (for reference, MIC_{90} is indicated in parenthesis) were 50.00(6.25), 3.13(6.25), 25.00(25.00), 3.13(12.50), 3.13(3.13), 25,00(25.00), and 0.78(3.13)μg/ml, respectively. From these results, *E coli* and *S typhimurium* were considered to have some resistance to available antibacterials exercised in this experiment, which confirmed our previous report[19]. The combined in vitro effect of two antibiotics can be evaluated by agar diffusion method, paper strip method, rate of killing method, cellophane transfer test, velvet pad replica plating method, chessboard titration, and checkerboard titration[17,18,20]. Recently, checkerboard method was highly recommended from the standpoints of its economy, reproducibility, and precision. In this experiment, we used this method in order to analyze the combination effects of two antibacterials. In checkerboard method the degree of combined effect can be expressed as FIC index[11,21]. The criteria of a combination of two antibiotics to act synergistically, additively, or antagonistically are based on the FIC index. Based on this criteria, the combination of NFX and colistin against *E coli* K88ac and *S typhimurium* 1926 showed synergistic effects. Meanwhile, the combination of NFX with trimethoprim, amikacin, gentamicin against *E coli* K88ac indicated the addition. The same addition effects were shown against *S typhimurium* 1926 but gentamicin showing addition/antagonism. In the case of tetracycline, the combination with NFX always showed antagonistic effects against both *E coli* K88ac and *S typhyimurium* 1926. The results of checkerboard titrations are listed in Table 1 and Table 2, Table 3. shows the varying combination ratio of NFX and colistin in terms of FIC indexes, which could be used to search for optimal combination ratios of NFX and colistin. Despite syn-

ergistic effects shown in the combination of the two antibiotics, we found there were antagonistic phenomena against *E coli* K88ac and *S typhymurium* 1926 in the ratio of 1 part of NFX and 0.08 part of colistin. Therefore, it is of prime importance to select optimal combination ratio between two antibiotics. All MIC determinations were performed twice on different days, and the results were averaged to achieve and assigned MIC for a given organism-antimicrobial agent combination. When duplicate MICs varied by one two-fold concentration increment, the higher MIC was taken as the assigned MIC.

The persistent suppression of bacterial growth after short antimicrobial growth after short antimicrobial exposure is called the postantibiotics effect(PAE)[22]. By definition, there should be no inhibitory concentrations of antimicrobial agent left when the PAE starts. A long PAE provides the administrating antimicrobials to have the potential for longer intervals between doses[10,22]. This fact has stimulated intensified research concerning the PAE phenomenon during the last decade[23-25]. In other reported data, PAE of NFX was performed by viable counts on gram positive and gram negative bacteria, resulted in 2.4 and 2.2 hr, respectively[2]. These results are similar to that of our experiments.

Conclusions

In order to evaluate the antibacterial activity of NFX against veterinary pathogenic bacteria, we determined minimal inhibition concentrations(MICs), fractional inhibition concentrations(FICs) and postantibiotic effects(PAE) using these organisms. Of the *Escherchia coli*(89) tested, 90% were inhibited by 0.02ug of NFX per ml. The highest MIC_{90} of NFX obtained was 4μg/ml for gram-positive organisms, *Streptococcus* spp.(24) and *Staphylococcus aureus*(34). The MICs for 50%(MIC_{50}) and 90%(MIC_{90}) of NFX against *Salmonella* spp.(56) were 0.2 to 0.2ug/ml, respectively. When it was compared with other antibacterials, NFX had greater activity than those of the other antibacterials; the MIC_{90}s for the Enterobacteriae were 0.02μg of amikacin per ml; 0.02μg of colistin per ml; and 8 or 4μg of penicillin or cephazolin per ml. NFX was less active against *S auerus*; 99% of the strains were inhibited by 4μg/ml. NFX was active compound against *E coli*, with 0.02μg/ml inhibiting 90% of the strains and 0.2 4μg/ml inhibiting 90% of *Salmonella* spp. In the case of the combined effects of NFX and colistin against *E coli*, MIC of norfloxacin was 0.08μg/ml which was reduced from 0.63μg/ml when used alone, with that of colistin being 0.78μg/ml from 3.13μg/ml. The FIC indexes for combination of norfloxacin with colistin, gentamicin, trimethoprim, amikacin, penicillin, tylosin and tetracycline against *E coli* K88ac were 0.38, 0.52, 0.56, 0.63, 1.00, 1.02 and 1.49, respectively. The FIC indexes for combination of NFX with the same antibacterials against *S typhimurium* 1926 were similar to the values against *E coli* K88ac. In addition, PAE of NFX was 0.90~1.02h against *E coli* K88ac and 1.58~1.99h against *S aureus* R-209.

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