Effect of Enrofloxacin-Na against Pathogens Related to the Respiratory and Alimentary Diseases in Suckling and Weanling Piglets

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ABSTRACT. A field trial was conducted to evaluate effect of enrofloxacin-Na against pathogens related to the respiratory and alimentary diseases in eighty suckling piglets (6–7 days old) and eighty weanling piglets (5–6 weeks old). Respective twenty of the suckling and weanling piglets were assigned to each of 4 experimental groups; control (non-treated), clinical injection dose (CID), 2x clinical injection dose (2CID), and premix. A 0.05 ml (2.5 mg) of enrofloxacin-Na injection (5% solution, 1 ml) per kg body weight of piglets as CID was injected intramuscularly for 3 days and the clinical signs were observed for 9 days. The premix (150 ppm) of enrofloxacin-Na was administered with feed for 7 days ad libitum and the clinical signs were observed for 13 days. The enrofloxacin-Na-treated piglets showed a higher increase in body weight and a lower feed per gain than the control piglets. In addition, the treatment of enrofloxacin-Na, regardless of the route of administration, decreased the incidence rate of diarrhea in suckling piglets and respiratory symptoms in weanling piglets. The isolation index of E. coli and Cl. perfringens during the treatment periods was also lowered by the enrofloxacin-Na treatment in both suckling and weanling piglets. The antibiotics was also evaluated as safe locally and whole bodily as treated by injection or feeding. These results indicate that the newly developed antibiotics, enrofloxacin-Na, is very useful for the prevention and therapy of swine diseases in the pig industry.

KEY WORDS: bacterial pathogen, diarrhea, enrofloxacin-Na, respiratory and alimentary symptoms, swine.

Enrofloxacin is a fluoroquinoline antimicrobial agent developed exclusively for use in veterinary medicine [7]. Enrofloxacin has high antimicrobial activity in vitro against a wide range of gram-negative and gram-positive bacteria [13]. It is easily absorbed in the intestinal tract and transported to body compartments [6, 10]. The quinoline carboxylic acid derivatives impair bacterial DNA gyrase, an enzyme that plays a major role in the replication of DNA. Inhibition of the enzyme leads to functional disturbances with blockage of certain stages in the synthesis, resulting in the death of bacteria [2]. Enrofloxacin inhibits DNA gyrase by interacting with DNA. The antimicrobial properties of enrofloxacin indicate that it may be advantageous to use in pigs for bronchopneumonia, enzootic pneumonia, colibacillosis, and salmonellosis [5, 11].

The fluoroquinolones containing F or a piparazine ring are a class of antimicrobial agents derived from naphthyridine nalidixic acid [4]. This class generally exhibits high bactericidal activity against gram-negative bacilli, moderate bactericidal activity against staphylococci, and fair to poor bactericidal activity against streptococci and anaerobes [4]. Recently, LG Chemical Ltd. (Seoul, Korea) has developed a new drug, enrofloxacin-Na, by adding Na to enrofloxacin free acid. Solubility of the antibiotic was increased by the substitution of H with Na. An in vitro efficacy test showed that enrofloxacin-Na has a strong growth inhibition against wild bacterial strains isolated from cattle, pigs, and poultry [13]. In this study, we evaluated the clinical in vivo efficacy and safety of the enrofloxacin-Na in the field of the pig industry.

MATERIALS AND METHODS

Materials: Enrofloxacin-Na (5%, injectable solution) and its premix (2.5%, feed form) were obtained from LG Chemical Ltd. (Seoul, Korea). According to manufacturer’s directions, 0.05 ml (2.5 mg) of enrofloxacin-Na per kg body weight of piglets was injected intramuscularly for 3 days and the premix (150 ppm) was administered with feed for 7 days.

Animals: Eighty suckling piglets (6–7 days old) weighing 2.59 ± 0.47 kg and eighty weanling piglets (5–6 weeks old) weighing 11.89 ± 2.80 kg were used for this study. Twenty (2 pens × 10 heads/pen) of the suckling or weanling piglets were assigned to each of 4 experimental groups; control (non-treated), clinical injection dose (CID), 2x clinical injection dose (2xCID), and premix. Each animal was tagged with individual number.

Sample preparation: Using a BBL culturette (Becton Dickinson Microbiology Systems, Cockeysville, Maryland, U.S.A.), anal and nasal samples were taken from experimental pigs on days 0, 3, 6, and 9 (additionally, day 13 for the control and premix groups), and they were transported in an ice box (4°C) to the Laboratory of Infectious Diseases, College of Veterinary Medicine, Seoul National University (Suwon, Korea). The samples were inoculated in appropriate media for culture.

Feed intake and body weight measurement: Feed intake and body weight were measured on days 0, 3, 6, and 9 (additionally, day 13 for the control and premix groups). The
amount of feed intake during the experimental period intervals was calculated from “Total supplied amount of feed - Remained amount of feed”. The efficiency of feed for the increase of body weight was expressed as feed per gain (FPG).

Clinical observation: The clinical respiratory (coughing) and alimentary (diarrhea) signs were observed daily for 10 days for CID and 2xCID groups and 14 days for premix and control groups.

Bacterial culture test: The presence of bacteria including Escherichia coli, Clostridium perfringens, Salmonella spp., Bordetella bronchiseptica, Pasteurella multocida, and Actinobacillus pleuropneumoniae was determined after cultivating with appropriate media. EMB (Difco Co.) for E. coli, blood agar (KoMed Co.) for Cl. perfringens, and tetraionate broth containing 2% iodine solution for Salmonella spp. were used. After the samples were incubated for 2 days at 42°C in tetraionate broth, followed by the culture on MacConkey, XLD, SS and Brilliant Green agar (Difco Co.) for 24 hr at 37°C for Salmonella spp. Cl. perfringens were cultured under an anaerobic condition with GasPak (BBL). Also, G20G agar (Bacitracine 20 g, NaCl 5 g, Bromthymol blue 40 mg, Furaladone 0.5 mg, gentamicin 0.5 mg, penicillin 20 mg, Fungizone 20 mg, glucose 10 g, lactose 10 g/distilled water) for B. bronchiseptica, chocolate agar (KoMed Co.) for A. pleuropneumoniae, and blood agar (KoMed Co.) for P. multocida were used for the culture for 24 hr at 37°C. A. pleuropneumoniae was cultured under a 5% CO2 pressure. Characteristic colonies were selected, gram-stained, and examined for biochemical characteristics including the productivity of H2S, catalase, oxidase and indole, and citrate utilization according to Bergey’s manual. The bacteria were identified using an auto-identification apparatus, Vitek system (BioMerius Vitek Inc., Hazewood, MO, U.S.A.). The change in bacterial growth in a piglet was determined using a standard of 5 grades, based on the bacterial number per plate (index 1 was less than 101 cells, indexes 2 to 4 were from 102 to 105 cells, respectively and index 5 was more than 106 cells). The group parameter was the sum of the individual piglet parameter. Meanwhile, the change of Salmonella spp. in piglets was expressed as the number of piglets with the detection of Salmonella spp.

Safety evaluation: After injection of enrofloxacin-Na at the dose (2xCID) of 5 mg/kg body weight using 20 suckling and 20 weanling piglets, local responses such as swelling, redness, and inflammation and systematic responses such as vomiting, emaciation, hair change, and feed intake were investigated throughout the experimental period.

Lesions in lung: Among eight piglets manifesting the respiratory symptoms such as coughing, two piglets were assigned to each of 4 experimental groups including CID group, 2xCID group, premix group, and control. After treatment for 7 days, lesions in the lung of the piglets were gross-pathologically examined.

Statistical analysis: The significance between the control group and the treatment groups was analyzed by the student’s t-test.

### RESULTS

Feed intake and feed per gain (FPG): In both suckling and weanling piglets, the feed intake was similar in all experimental groups throughout the experimental periods. The FPGs of suckling piglets in CID group, 2xCID group, premix group, and control group were 0.249, 0.279, 0.413, and 0.379, respectively. The injection groups showed a lower FPG than the control group, whereas the premix group showed a slightly higher FPG than the control group (Table 1). The FPGs of weanling piglets in the CID group, 2xCID group, premix group, and control group were 1.278, 1.174, 1.171, and 1.290, respectively. All the drug-treated groups showed a lower FPG than the control group, where the premix group showed the lowest FPG among them (Table 1).

Body weight change: Although there were no significant changes in the body weights of suckling piglets, a markedly increase in the body weights was observed in the CID group and slightly increases were found in both the 2xCID group and the premix group (Fig. 1a). In weanling piglets, there were no differences in the body weights of piglets between the experimental groups on day 3. After then, the body weights in the drug-treated groups were higher than the control group, although there was no significant difference between them (Fig. 1b).

Clinical signs: No animals in all experimental groups died during experimental period. Diarrhea and respiratory symptoms were mainly examined during the experimental periods. Generally diarrhea occurred in suckling piglets while respiratory symptoms occurred in weanling piglets. The diarrhea of suckling pigs was lasting to the end of the experiment, if occurred. The diarrhea in suckling pigs occurred with a low incidence rate of 0–10% until day 8 in the CID group and until day 5 in the 2xCID group (Fig. 2a). After then, the incidence rates were increased to 30–40%. In the premix group, the incidence rate was 25% at day 2 and it was continuously increased to 50% at days 8–9. In the control group, the diarrhea incidence rate was 25% at day 3 and 80% at day 6. However, the incidence rate in the control group was decreased with time thereafter, representing less than 50% at day 10 (Fig. 2a). These result suggested that the i.m. injection of enrofloxacin-Na decreased the incidence rate of diarr-
rhea in suckling piglets up to 70% and that the premix feeding also decreased the rate up to 30%.

In weanling piglets, the control group showed 20–40% incidence rates of respiratory symptoms during the experimental periods (Fig. 2b). However, the administration of enrofloxacin-Na decreased the incidence rate of the respiratory symptoms by 20–25% (Fig. 2b). In the control group, the hair was rough and respiratory symptoms frequently occurred at the later periods of the experiment. As compared with the control group, the administration of enrofloxacin-Na...
in suckling piglets and weanling piglets evidently decreased the incidence of diseases (Fig. 2).

Change of etiological pathogens in alimentary and respiratory diseases: In suckling piglets, the injection groups showed a sharp decrease in the growth of *E. coli* until day 3, a constant growth during days 3–6, and an increase in the growth thereafter, compared to the premix and control groups (Fig. 3a). The premix group showed a trend of slight decrease in the growth of *E. coli* until day 6, a sharp decrease of the growth during 6–9, and the maintenance of the growth thereafter. In weanling piglets, the injection groups showed a constant decrease in the growth of *E. coli* and a decrease in the growth of *Clostridium perfringens* until day 6, and the maintenance of the growth thereafter. The premix group showed a trend of slight decrease in the growth of *E. coli* until day 9, and the maintenance of the growth thereafter. The control group showed a sharp decrease in the growth of *E. coli* and a decrease in the growth of *Clostridium perfringens* until day 6, and the maintenance of the growth thereafter.

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**Fig. 3.** Isolation index of *Escherichia coli* in (a) suckling piglets and (b) weanling piglets.

**Fig. 4.** Isolation index of *Clostridium perfringens* in (a) suckling piglets and (b) weanling piglets.
growth pace thereafter. The control group showed a slight increase of the growth of *E. coli* until day 3, and a slight decrease during days 3–6, a sharp decrease during days 6–9, and the maintenance of the growth pace after that. In weanling piglets, all the enrofloxacin-Na-treated groups showed a sharp decrease in the growth of *E. coli* until day 3, a constant growth during days 3–6, and an increase in the growth thereafter (Fig. 3b). The control group showed a slight decrease during experimental period.

In suckling piglets, the injection groups showed a sharp decrease in the growth of *Cl. perfringens* until day 3, a slight increased growth during days 3–6, and again a decrease in the growth thereafter (Fig. 4a). The premix group showed a trend of sharp decrease in the growth of *Cl. perfringens* until day 3 and a slight decrease of the growth thereafter. The control group showed no change of the growth of *Cl. perfringens* until day 3, and a sharp decrease during days 3–6, a slight decrease after that. In weanling piglets, the enrofloxacin-Na-treated groups including CID, 2xCID and premix showed a sharp decrease in the growth of *Cl. perfringens* until day 3, a constant growth or a slight increase of the growth thereafter (Fig. 4b). The control group showed a slight decrease in the growth of *Cl. perfringens* until day 9 and a slight increase thereafter.

Meanwhile, there was no specific difference in the growth of *B. bronchiseptica, A. pleuropneumoniae*, and *P. multocida* associated with respiratory symptoms between the control and the drug-treated groups, because the isolation rates of respiratory symptom-related bacteria were very low in all experimental groups.

The presence rate of *Salmonella* spp. in weanling piglets was lower than that in suckling piglets. The administration of the enrofloxacin-Na might lower the isolation rate of *Salmonella* spp. (data not shown).

**Safety evaluation:** No specific changes such as inflammation and necrosis of local injection site, feed intake, and hair condition were found in all experimental groups.

**Lesions in lung:** There were no specific lesions in the lung of the weanling piglets in all experimental groups. This result may be related to no detection of respiratory symptom-related bacteria.

**DISCUSSION**

Chronic wasting diseases including colibacillosis, enterotoxemia, salmonellosis, pleuropneumonia, pneumatic pasteurellosis, and atrophic rhinitis are still prevalent and cause a severe economic loss in the pig industry. To prevent or treat against these bacterial infections, many antibiotics have been developed. Baytril, enrofloxacin injection, has been mainly used for the purpose of prevention and treatment of these diseases [5, 8]. In recent, LG Chemical Company developed enrofloxacin-Na of which the solubility is higher than that of Baytril. The efficacy of the antibiotics has already been confirmed by *in vitro* tests using many etiological pathogens for swine diseases [13]. In this study, the effect of enrofloxacin-Na against pathogens related to the respiratory and alimentary diseases in piglets was investigated and the safety of the enrofloxacin-Na was evaluated.

Both suckling and weanling piglets showed a little higher feed intake in the premix group than the control group. This result indicates that the feeding trial does not decrease appetite in the piglets. In addition, FPG of the drug-treated groups in weanling piglets was higher than that of control group. However, a slight decrease of FPG in suckling piglets may be attributed to a decreased feed intake.

In suckling piglets, the injection of enrofloxacin-Na seemed to be more effective in preventing the incidence of diarrhea than the feeding of premix, probably resulting from a lower feed intake (corresponding to a lower intake of the drug) in the premix group. Clinical signs after stopping administration of enrofloxacin-Na were prevalent, maybe due to the change of bacterial growth following excretion of the drug [1]. In this study, suckling piglets showed mainly diarrhea while weanling piglets showed mainly respiratory symptoms. The incidence rate of respiratory symptoms in weanling piglets was lower than the incidence rate of diarrhea in suckling piglets. However, the treatment of enrofloxacin-Na lowered both the incidence rates in the piglets. This result suggests that the efficacy of enrofloxacin-Na is equal to that of enrofloxacin [3].

In suckling piglets, the changes in bacterial growth of *E. coli* and *Cl. perfringens* related to diarrhea were associated with treatment of enrofloxacin-Na. The fecal growth of the bacteria in weanling piglets was also associated with the treatment of enrofloxacin-Na, regardless of the incidence of diarrhea. These results indicate that the enrofloxacin-Na directly acts on the bacterial growth in the piglets. The administration of the drug also lowered the detection of *Salmonella* spp. in suckling pigs compared to the control group.

In this study, the respiratory symptom-related bacteria were not isolated. This result suggests that the bacteria may not be related to the incidence of respiratory symptoms or other etiological factors may be present. Meanwhile, we did not obtain a direct evidence for therapeutic effect of enrofloxacin-Na on *A. pleuropneumoniae* and *P. multocida*, probably due to several factors including timing of experiment, age of animals, breeding condition, etc. However, enrofloxacin-Na was known to have an antibacterial effect against *A. pleuropneumoniae* [9, 12, 13]. Therefore, the effectiveness of the drug on the bacteria can not be excluded.

In conclusion, enrofloxacin-Na is a very useful antimicrobial agent for the prevention and therapy of swine diseases in the pig industry, regardless of the route of administration. In addition, it has beneficial effects on body weight increase and feed-per-gain in piglets.

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