Comparison of the Efficacy of Enrofloxacin Against *Escherichia coli* or *Pasteurella multocida* Infection in Chickens

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Abstract

This study compared the efficacy of enrofloxacin, given immediately or 6 hrs after infection against *Escherichia coli* or *Pasteurella multocida*. In the first experiment, thirty, 12-week-old male layer chickens were divided into 3 groups of 10 each. Chickens in group 1, 2 and 3 were infected with *E. coli* serotype O78 by left thoracic air sac injection. Group 1 was given enrofloxacin (10 mg/kg), dissolved in drinking water immediately receiving *E. coli*. Group 2 was given enrofloxacin 6 hrs after receiving *E. coli*. Group 3 served as a positive control. In the second experiment, twenty, 16-week-old male layer chickens were divided into 3 groups. Groups 1 and 2 have 7 chickens each and group 3 has 6 chickens. Chickens in group 1, 2 and 3 were infected with *Pasteurella multocida* by thigh muscle injection. Group 1 was given enrofloxacin (10 mg/kg), dissolved in drinking water immediately after receiving *P. multocida*. Group 2 was given enrofloxacin 6 hrs after receiving *P. multocida*. Group 3 served as a positive control. The result revealed that enrofloxacin has efficacy to treat colibacillosis and fowl cholera. Chickens immediately treated with enrofloxacin showed lower mortality both of colibacillosis and fowl cholera infection. The lesion scores of airsaculitis, pericarditis and perihepatitis in the chickens immediately treated with enrofloxacin showed lower than the chickens treated 6 hrs later.

Keywords: Colibacillosis, enrofloxacin, fowl cholera, treatment

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บทคัดย่อ

ปุ_IListที่พบในประเทศของเอเชียที่มีผลกระทบต่อการติดเชื้อ เอสเชอริเชีย โคไล และพาสจูเรลลา มัลโตซิดา ในไก่

วิชัย์ ระวิเวช 1 ปิยะรัตน์ อังรีดูนังต์ 2 พิชญา นิวัตร จันทรศิริพรชัย 3

การศึกษานี้มีวัตถุประสงค์ในการเปรียบเทียบประสิทธิภาพของเอนโรฟลอกซาซิน โดยการให้ยาทันที หรือให้ยา 6 ชั่วโมงหลังการติดเชื้อ เอสเชอริเชีย โคไล และพาสจูเรลลา มัลโตซิดา ในการทดลองแรก เบื้องหน้ากลุ่ม 12 สัตว์ที่ จำนวน 30 ตัว แบ่งออกเป็นกลุ่มละ 10 อีก 1 กลุ่ม ได้รับเอนโรฟลอกซาซิน 10 มก./ก.g. โดยการฉีดเข้ากล้ามเนื้อหน้าแข้งทันทีหลังจากได้รับเชื้อ 1. เป็นกลุ่มควบคุมบวก 3 ตัว 2. ได้รับเอนโรฟลอกซาซิน 6 ชั่วโมงหลังจากได้รับเชื้อ 3. ได้รับยาทันทีหลังจากได้รับเชื้อ ทั้ง 3 กลุ่ม มีประสิทธิภาพในการป้องกันโรคโคไลบาซิลโลซิส (P. multocida) ในไก่ที่ได้รับเอนโรฟลอกซาซินทันทีมีอัตราการตายต่ำกว่าต่ำกว่ากลุ่มที่ได้รับการรักษาหลัง 6 ชั่วโมง

คำสำคัญ: โคไล, เอนโรฟลอกซาซิน, 的效果 และการควบคุม

1 หน่วยปฏิบัติการวิจัยสุขภาพสัตว์ปีก 2 ภาควิชาเภสัชวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย 30 ถ.อัษฎาจินดา ปทุมวัน กรุงเทพฯ 10330 ประเทศไทย

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Introduction

Escherichia coli is a normal organism of the gut microflora of the chickens, and most serotypes are not pathogenic. However, a number of serotypes of E. coli are pathogenic, they are inhaled into the respiratory tract and are probably the most frequent and economically significant cause of bacterial disease known as colibacillosis in chickens. The most common form of coli septicemia is characterized by an infection of the air sacs. The air sacs of poultry lie outside of the lungs in the single body cavity. These expandable air sacs function principally as airways; when they are forced to expand and contract air is moved through the relatively incompressible lungs. Air sacculitis is often associated with bacteremia, septicaemia, pericarditis and perihepatitis, usually resulting in approximately 5% mortality in commercial situations (Wray et al., 1996).

Fowl cholera caused by the Gram-negative, non-motile, coccobacillary bacterium Pasteurella multocida has been known to occur in a variety of wild and domestic birds and to cause major economic losses in the poultry industry. Death results primarily from peracute and acute septicaemia and less frequently from the chronic and localized form of the disease.

Infections of chickens with E. coli or P. multocida can be prevented and controlled by antibiotics. However, a serious drawback of the prophylactic use of such chemotherapy is the development of resistance in avian pathogenic E. coli (APEC) to most antibiotic (Chansiripornchai et al., 1995). Genes that are located on plasmids often encode resistance to antibiotics. These plasmids easily spread through bacterial populations, which lead to the spread of resistance, so rendering the drugs ineffective. One group of antibiotics, the fluoroquinolones, is less likely to undergo this fate, because they inhibit the synthesis of DNA gyrase A, which is essential for duplication of plasmid (Glisson, 1994; Chansiripornchai et al., 1995).

Fluoroquinolones are broad-spectrum drugs with a bactericidal activity and their uses for the prevention and control of avian infections with E. coli and P. multocida. Enrofloxacin is antibiotics belonging to the fluoroquinolone class of compounds. This drug is more potent than any earlier analogues, has a broad spectrum of activity and drug resistant bacteria are induced less frequently (Wolfson and Hooper, 1988).

The objectives of the current experiment were to compare the efficacy of enrofloxacin against E. coli and P. multocida infection in the different times of drug application.
Materials and Methods

Chickens: Unvaccinated male Babcock layer chickens were obtained on the day of hatching from a commercial hatchery. The chickens were fed *ad libitum* before and during the experiments. At the onset of the experiments there was no statistically significant difference in average weight between the experimental groups. Chickens in experiments 1 and 2 were brought from different batches of the same hatchery so that there might be differences among chickens in each experiment. The guidelines and legislative regulations on the use of animals for scientific purposes of Chulalongkorn University, Bangkok, Thailand were followed as is certified in permission No.0831068.

Bacterial strains: The chickens were challenged with an *E. coli* strain of serotype O78 that was originally isolated from the diseased air sacs of a chick with a field case of colisepticemia. Sekizaki et al. (1989) showed that this *E. coli* strain produced high mortality in a very short time. In the first experiments, we injected 1.0 ml of the *E. coli* suspension, containing 10^6 cfu/ml. *E. coli* suspension was injected into the left caudal thoracic air sac of infected chickens. In the 2nd experiment, the chickens were challenged with *P. multocida* strain 8A that was also originally isolated from the liver of the fowl cholera infection chicken. One ml of *P. multocida* suspension, containing 10^5 cfu/ml was injected into each bird. *P. multocida* suspension was injected into the left thigh of infected chickens.

Medication: Enrofloxacin (Quinoxine-10, F.E. Pharma Co. Ltd., Thailand) was administered to the chickens in their drinking water at the concentrations specified in the experimental designs. The water intake for birds had been measured before the experiments were performed. So, we knew approximately the water intake for our experimental designs. The water intake for birds had been measured before the experiments were performed. At the onset of the experiments, the chickens were given immediately or 6 hrs later in the drinking water immediately after the experiments started. In the experimental groups, enrofloxacin was given immediately or 6 hrs later in the drinking water of chickens for twice a day. The enrofloxacin was given to the chickens within 2 hrs for 5 consecutive days. The morbidity and mortality were recorded for the chickens within 2 hrs for 5 consecutive days. The morbidity and mortality were recorded for the experimental groups in both experiments were included in order to compare the data within each experiment. The bacteria were challenged immediately after the experiments started. In the experimental groups, enrofloxacin was given immediately or 6 hrs later in the drinking water of chickens for twice a day. The enrofloxacin was given to the chickens within 2 hrs for 5 consecutive days. The morbidity and mortality were recorded for the seven and three days following infection of the chickens with *E. coli* and *P. multocida*, respectively. The pathological lesions in the dead chickens were investigated at necropsy.

Experiment 1: To study the efficacy of enrofloxacin given immediately or 6 hrs later in the drinking water of chickens that were challenged with *E. coli*. Thirty, 12-week-old male layer chickens were divided into three groups of 10 each. Chickens in group 1, 2 and 3 were infected with *E. coli* serotype O78 by left thoracic air sac injection. Group 1 was given enrofloxacin (10 mg/kg), dissolved in drinking water immediately after receiving *E. coli*. Group 2 was given enrofloxacin 6 hrs after receiving *E. coli*. Group 3 served as a positive control.

Experiment 2: To study the efficacy of enrofloxacin given immediately or 6 hrs later in the drinking water of chickens that were challenged with *P. multocida*. Twenty, 16-week-old male layer chickens were divided into three groups of 7, 7 and 6 chickens in each group, respectively. Chickens in groups 1, 2 and 3 were infected with *P. multocida* serotype 8A by left thigh muscles injection. Group 1 was given enrofloxacin (10 mg/kg), dissolved in drinking water immediately after receiving *P. multocida*. Group 2 was given enrofloxacin 6 hrs after receiving *P. multocida*. Group 3 served as a positive control.

Efficacy criteria and definitions: Mortality was defined as the number of birds that were killed or that died before the end of the trial. Morbidity was defined as the number of birds with either air sac, pericardium or perihepatic lesions. The air sacs, pericardial and perihepatic lesions of colisepticemia in each bird were scored. The air sac lesions of colisepticemia were scored according to Kleven et al. (1972) as follows: 0: no lesions, 1: cloudy air sacs, 2: air sac membranes are thickened, 3: “meaty” appearance of membranes, with large accumulations of a cheesy exudate confined to one air sac, 4: lesions with the same score as score 3 but with lesions in two or more air sacs. The pericardial lesions of colisepticemia were scored according to Charleston et al. (1998) as follows: 0: no lesions, 1: extensive clear or cloudy fluid in the pericardium, 2: extensive fibrination in the pericardial cavity. The perihepatic lesions of colisepticemia were scored according to Charleston et al. (1998) as follows: 0: no visible lesions, 1: definite fibrin on the surface of the liver, 2: extensive fibrination, adhesions, liver swelling and necrosis.

Statistical analysis: ANOVA and Duncan multiple range tests were used for the statistical comparison of the body weight. The mortality, morbidity and the lesion scores were analysed by Chi-square and Mann-Whitney U test, respectively. SPSS for Windows was used for statistical analysis.

Results

Efficacy of enrofloxacin treatments against *E. coli* infection: The chickens in groups 1 and 2 which received enrofloxacin immediately and 6 hrs after challenged with *E. coli* revealed less morbidity and mortality comparing to the positive control group. The mortality of chickens in group 1 was statistically significant less than (*p*<0.05) that of the control group. The post mortem pathology of the *E. coli* challenged chickens were airsacculitis, fibrinopurulent perihepatitis, fibrinopurulent pericarditis and peritonitis. These typical clinical symptoms were similar to the enrofloxacin treated group and the non treated group, but the lesions of airsacculitis, fibrinopurulent pericarditis, and fibrinopurulent perihepatitis were less in the treatment group. Also, the lesion scores of airsacs, pericardium and livers of chickens in group 1 was statistically significant less than (*p*<0.05) that of the control group (Table 1).
Efficacies of enrofloxacin treatments against P. multocida infection: The chickens in groups 1 and 2 which received enrofloxacin immediately and 6 hrs after challenged with P. multocida revealed less morbidity and mortality comparing to the positive control group. The mortality of chickens in group 1 and 2 was statistically significant less than (p<0.05) that of the positive control group. All chickens in the positive control group were died. The post mortem pathology of the P. multocida challenged chickens were generalized hemorrhage at the various muscles especially heart and thigh muscle. The hemorrhage also found at the coronary fat. All of chickens died within one day after the challenge showed typical lesions of hemorrhage. The typical clinical symptoms were similar to the enrofloxacin treated group and the non-treated group, but the hemorrhage was less in the treatment group (Table 1).

Discussion

In the experiment, chickens were challenge with E. coli serotype O78 produced 50% mortality and 10% morbidity in the positive control group. The mortality rate in the experiment is much higher than that of the field cases. In the field cases, the mortality level is 0.25% in the primary and increase to 1% after infected for 5 day and can rise up to 10% (Shane et al., 1981; Wray et al., 1996). In the experimental model, 5% mortality was found after challenge with less than 10^4 CFU of E. coli, but it increased to 50% when the challenge dose was increased to 10^6 CFU (Dunnington et al., 1991). This data is according to our infection model because the high mortality was observed among the infected, non-medicated birds.

Death losses from fowl cholera in chickens usually occur in laying flocks, because birds of this age are more susceptible than younger chickens. Chickens less than 16 weeks of age generally are resistant to the fowl cholera infection. In the P. multocida infection model used in this experiment produced 100% mortality in non medicated birds. This level is much higher than the 50% mortality in the field that may occur by high challenge dose (10^6 CFU). Under experimental conditions, 90-100% of mature chickens exposed by swabbing the palatine cleft may die within 24-48 hrs. The mortality was 35-45%, depending on the strain of P. multocida used, but only 10-20% usually dies within a 2 weeks period when exposed by contact with infected birds (Pritchett et al., 1930).

In both experiments showed that the immediately enrofloxacin treatment and 6 hrs treatment after infection of E. coli and P. multocida were not significantly reduce pathological lesions and mortality; indeed, there was a trend for lower mortality and fewer pathological lesions in the bird was treated with enrofloxacin immediately after infection than in the bird was treated with enrofloxacin 6 hrs later. The general lesions developed by inoculation APEC or bacteria-free culture filtrates into the air sac. Airsaculitis occurs within 1.5 hrs. Bacteremia and pericarditis develop within 6 hrs and most mortality occurs during the first 5 day (Derosa et al., 1992). Thus the bird was treated with enrofloxacin 6 hrs after E. coli infection had tend to reduce lesion and mortality less than the bird was treated with enrofloxacin immediately because the lesion had occurred before receiving drug. In the P. multocida model the bird was treated with enrofloxacin immediately infection had not significant lower mortalities than the infected birds that treated with enrofloxacin 6 hrs after infection. The result of this study also accorded to the findings of Chansiripornchai (2007) that Chickens received antibiotics 1 hr before challenges, immediately after challenges or 1 hr after challenges. The result revealed that enrofloxacin has efficacy for prevention and treatment of fowl cholera. No death chickens had been found in these 3 enrofloxacin treated groups. However, FCR of the chickens received antibiotics before the challenges tended to be better than those of chickens received antibiotics immediately after challenges or 1 hr after challenges. The current experiment revealed the efficacy of enrofloxacin to treat colibacillosis and fowl cholera, the results may cause by the merit of rapid tissue penetration of enrofloxacin. A time to maximum (Tmax) of enrofloxacin (Baytril®) in serum, lung and muscle is 0.5, 2 and 2 hrs, respectively. Also, a maximum tissue concentration (Cmax) of enrofloxacin (Baytril®) in serum, lung and muscle is 0.77, 1.76 and 1.75 µg/ml or µg/g, respectively (Franz, 2006). The Tmax of enrofloxacin is faster than the incubation period of colibacillosis and fowl cholera around 24 hrs (Chansiripornchai, 2007; 2009). A minimum inhibition concentration of the E. coli isolate is 0.5 µg/ml (data not shown), the level is still lower than the Cmax level of enrofloxacin in serum, lung and muscle.

In conclusion, the pathological lesions and mortality of the E. coli infected birds which immediately treated with enrofloxacin after infection had not significant lower than those of the infected

Table 1 Pathological parameters of chickens infected with E. coli and P. multocida.

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<thead>
<tr>
<th>Group</th>
<th>E. coli infection</th>
<th>P. multocida infection</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Morbidity</td>
<td>Mortality</td>
</tr>
<tr>
<td>1</td>
<td>0/10^6</td>
<td>0/10^b</td>
</tr>
<tr>
<td>2</td>
<td>0/10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3/10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>1/10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5/10&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a</sup> The superscripts that differed in each column have significantly different at confidential 95% (p<0.05).
birds that treated with enrofloxacin 6 hrs after infection. The mortality of the \textit{P. multocida} infected birds with immediately treated with enrofloxacin infection had not significant lower than those of the infected birds that treated with enrofloxacin 6 hrs after infection. But the treatment of enrofloxacin would provide the less morbidity, mortality, clinical findings or pathological findings compared to the non-treatment group.

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\textbf{References}


