DANOFLOXACIN AS AN EFFICIENT ANTIMYCOPLASMAL AGENT

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Abstract

The efficacy of Danofloxacin in treatment and prophylaxis of experimentally infected chicks was tested. One hundred Hubbard chicks were divided into five groups of twenty each. First group was infected at 15 days old with virulent strain of Mycoplasma gallisepticum (MG) 5-6 then, treated with Danofloxacin (500ppm) one day after the appearance of the symptoms. Second group took a prophylactic dose for three days then, it was challenged with 5-6, followed by treatment after 24 hours from the appearance of symptoms. Third group was dosed with Danofloxacin prophylactically without infection for 7 days and considered as residue group. Fourth group was used as negative control (infected, untreated). Fifth group was positive-control (infected, untreated).

The results revealed that the second group (prophylactic then infected) gave more body weight and internal organs weight gain than the first (infected then treated) and third (residue) groups which both gave nearly same results.

Clinical symptoms were severe in the fifth and first groups accompanied with high mortality rate (80, 25%), respectively. All isolation of M. gallisepticum was successful in first (infected, treated), second (infected, prophylactic) and fifth (positive-control) groups.

Haemagglutination-inhibition (HI) titer was high in the first group during the first week after treatment, then, lowered by the second week. Meanwhile, HI titer of the second group was lower in first and second weeks after treatment than the first group, while, the residue and negative-control groups (3,4) showed a very high titer by the second week.

INTRODUCTION

For many years, workers have reported numerous attempts to treat mycoplasmosis in chickens and turkeys with various drugs and antibiotics. No substance has yet been found which completely could eradicate the pathogenic
organisms from all birds. Clinical improvement, better weight gains and feed conversion are often accomplished, and the majority of secondary bacteria, etc., were eliminated; but frequently M. gallisepticum remained latent in the treated birds, ready to infect fresh susceptible birds or to re-invade the original birds when their resistance or immunity became again lowered (Newnham, 1963). The problem of drug resistance also comes into the picture. M. gallisepticum became resistant to streptomycin extremely rapidly (Dornemuth, 1960 and Osburn et al. 1960), and also to the tetracyclines which are often included at a low level in some proprietary diets (Newnham, 1963). However, some resistance could be detected due to chromosomal mutation (Jordan, 1981, christiansson and March, 1983).

So, many efforts are required to overcome the problem of drug resistance and this can be done by using new antimicrobials. Fluoroquinolones are a relatively new class of potent synthetic agents, with a broad spectrum antibacterial activity, including strain resistant to many other antibacterial agents (Neer, 1988, Vansaebe and Percival, 1991 and Guay, 1992). The informations about the in vitro and/or in vivo activity of Danofloxacin against M. gallisepticum are limited (Jordan et al., 1983, Bradbury et al., 1994).

The present work aimed to study the efficacy of Danofloxacin with regard to:

- The control and treatment of the infected chicks.
- Effect on gain weight, feed conversion, gross lesions, reisolation of and antibodies to M. gallisepticum.

**MATERIALS AND METHODS**

**Antimicrobial agent**: Danofloxacin was obtained as a pure powder from Pfizer Ltd. Company and was used as oral droppings (50 ppm).

**Chicks**: One hundred, Hubbard broiler chicks were obtained at one day old from Middle East Poultry Company. All birds were examined by clinical and laboratory methods according to Jordan and Kulasagaram (1968) to be sure that they were free from Mycoplasma. At age of two weeks, the chicks were weighed individually and allocated at random into five groups of twenty birds. Each group had approximately the same average weight.

**Mycoplasma strain**: Virulent strain of Mycoplasma gallisepticum (S-6) was kindly supplied by Prof. Dr. Aly El-Dbeedy, Director General, Animal Health Research Institute, Giza.
**Culture Medium**: PPLO medium was prepared according to Hayflick (1965).

**Infection**: 0.1 ml (containing $10^9$ CFU) of *M. gallisepticum* (S-6) was sprayed into groups 1, 2 and 5 only. Chicks of groups 3 and 4 were sprayed with sterile diluents.

**Haemagglutination - inhibition test (HI)**: It was used as described by Meszaros (1964).

**Experimental Design**: One hundred chicks were divided into five groups:

1. First group: was infected with virulent strain of (S-6) and treated orally with Danofloxacin in drinking water (50 ppm) after 24 hours of starting symptoms of Mycoplasmia for four days, then, measuring the efficacy of Danofloxacin by changes in body weight, mortality rate, clinical symptoms, HI antibodies, and re-isolation of *M. gallisepticum*.

2. Second group: An attempt was made to protect or prevent Mycoplasmia in experimental chicks by giving the tested chicks Danofloxacin for three days, and after 24 hours the chicks were challenged with (S-6) then, treated for another four days to measure the efficacy as done with the first group.

3. Third group: the residual effect of Danofloxacin was studied by giving it for seven days, then, slaughtering the birds after other seven days.

4. Fourth group: was used as untreated negative control.

5. Fifth group: it took the infection and was left without any treatment as positive control. The experimental design was summarized in Table 1.

**Table 1. Summary of the experimental design for testing Danofloxacin Efficacy in chicks.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Design</th>
<th>Age/Day</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Infected, then</td>
<td>15</td>
<td>Infected with (S-6). Treated orally, Danofloxacin (50 ppm)</td>
</tr>
<tr>
<td></td>
<td>1 treated</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Prophylactic,</td>
<td>12</td>
<td>Orally, Danofloxacin (50 ppm). Treated, other 4 days.</td>
</tr>
<tr>
<td></td>
<td>infected, then</td>
<td>15</td>
<td>Infected with (S-6).</td>
</tr>
<tr>
<td></td>
<td>2 treated</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Residue</td>
<td>15</td>
<td>Orally, Danofloxacin (50 ppm/7 days).</td>
</tr>
<tr>
<td>4</td>
<td>Uninfected, untreated</td>
<td>15</td>
<td>Negative - Control.</td>
</tr>
<tr>
<td>5</td>
<td>Infected, untreated</td>
<td>15</td>
<td>Infected with S-6 (Positive - Control).</td>
</tr>
</tbody>
</table>
RESULTS

Danofoxacin was tested experimentally in 100 chicks to study its effect in the control and treatment of *M. gallisepticum* infection. The results of the experiment revealed more body weight and internal organs gain in the third and fourth group (690-710g (Residue and negative control groups), respectively. The body weight of the second group which took Danofoxacin before infection was higher than the first group which did not take it before infection (620, 515 g), respectively. The fifth infected group showed the least body weight (390 g). Similar findings were noticed on the mean weight of liver, kidneys and spleen. Table 2 summarized the changes in mean body weight and internal organs during three weeks period.

Clinical symptoms included congestion and oedema of lungs, turbidity of air-sacs and tracheal exudate. The signs were severe in the positive control group (5th) and the infected, then, treated one (1st) accompanied with mortality rate (60 and 25%), respectively. Neither symptoms nor deaths were noticed on the residue and negative-control groups 3rd and 4th.

The Haemagglutination-inhibition titer of the third and fourth groups were negative. The first group showed higher HI titer starting from the first week, then, lowered after second week of treatment (1:256 then 1:128, respectively). The titer in second prophylactic group (infected and treated) was lowered in the second week of treatment (1:128 then 1:64), respectively.

Table 2. Changes in mean body weight and internal organs in chicks tested for Danofoxacin efficacy.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Body weight / g</th>
<th>Mean Internal Organs / weight / g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st 2d 3d</td>
<td>Liver 1st 2d 3d</td>
</tr>
<tr>
<td>1</td>
<td>195 396 515</td>
<td>5.7 11.4 12</td>
</tr>
<tr>
<td>2</td>
<td>307 456 620</td>
<td>9.8 14.7 14</td>
</tr>
<tr>
<td>3</td>
<td>326 480 690</td>
<td>10.8 15 17.5</td>
</tr>
<tr>
<td>4</td>
<td>466 650 710</td>
<td>11 16.2 19.7</td>
</tr>
<tr>
<td>5</td>
<td>170 285 390</td>
<td>3.9 5 6.1</td>
</tr>
</tbody>
</table>

Re-isolation of Mycoplasma gallisepticum was successful in the first, second and fifth groups (infected then, treated; prophylactic then treated and positive control groups). Negative re-isolation results were obtained from third and fourth groups (residue and negative). Summary of these data was given in Table 3.
Table 3. Changes in clinical symptoms, mortality, HI titer, and reisolation of M. gallisepticum in chicks groups with Danofloxacin.

<table>
<thead>
<tr>
<th>Group</th>
<th>Clinical Signs</th>
<th>Mortality (Total 20)</th>
<th>HI titer (after treatment)*</th>
<th>Re-isolation of M. gallisepticum (Total 7 Bird) After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
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<td>3</td>
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<td>4</td>
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<tr>
<td>5</td>
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</tbody>
</table>

* Positive = 1: 64 and above (Meszaros, 1964).

DISCUSSION

Recently, a number of new Quinolone antibacterial drugs have been synthesized which showed improved anti-Mycoplasma activity over Quinolone compounds that were available hitherto (Kammam et al. 1989). Jordan et al. (1993) compared the efficacy of Danofloxacin and Tylosin in the control of M. gallisepticum infection in chicks, and found that the control of clinical signs and mortality was better, and more weight gain was obtained with Danofloxacin at 21 days after infection. Bradbury et al. (1994) carried out similar evaluation using Danofloxacin, a new Quinolone antimicrobial drug, against M. gallisepticum and M. synoviae and found that they were susceptible to it with minimal inhibitory concentration from 0.008 to 0.5 μg/ml.

Our results indicated that, protection based on the survival of chicks proved the efficacy of Danofloxacin in the control of mycoplasmal infection. As shown in Table 2, the mean body weight of treated group (1) was 515 g, prophylactic group (2) 620 g and reached 690 g in residue group (3). The untreated groups (4, 5) reached 710 g for the negative-control and 390 g for the positive-control group. The same results were noticed regarding the mean weight of the internal organs (liver, spleen and kidneys). The oral administration of Danofloxacin (50 ppm) minimized the depression in weight due to Mycoplasma infection, and reduced the severity of secondary infection. Similar observations were noticed by Gale et al. (1967) and Levett et al. (1980) who found that birds which were infected and treated with Tylosin and Tiamulin were heaver than the untreated ones. Kempf et al. (1992) studied the efficacy of Danofloxacin in the therapy of experimental mycoplasmosis in chicks and found that, treatment with Danofloxacin and Tylosin...
significantly decreased mortality rate and increased weight gain compared with un-medicated birds. Similar findings were reported by Tanner *et al.* (1993) and Migaki *et al.* (1993) revealed that the decrease in both the frequency of *M. gallisepticum* reisolation and seroconversion that occurred following Danofloxacin treatment indicated rapid elimination of organisms from the system.

Haemagglutination-inhibition titer in group 1 (infected, treated) and 2 (prophylactic, treated), as well as, the positive-control (infected, untreated) were positive. Similar findings were noticed by Fahey and Crawley, 1955 and Newnham. (1963). There is generally considerable individual variation in response among birds, towards both infection and HI antibody titer level. This experiment gave good picture of this variation, and of the misleading interpretation that can be obtained if only the clinical aspect of respiratory mycoplasmosis is considered. Even when birds appear clinically normal, the pathogens may still be found in the respiratory tract, but HI titers should give an indication of past or present infection. These results agreed with those of Fahey and Crawley (1955), Heishman *et al.* (1962) and Newnham (1963) who reported the lowering of HI titers in infected birds by administration of tetracycline in diet.

*Mycoplasma gallisepticum* was re-isolated from live birds till the end of experiment (groups 1,2,5). Thus, although treatment of infected birds resulted in better weight gain compared with those for untreated stock, flock infection still remained, and under the condition of the experiment the drug was not mycoplasmacidal. However, therapy for a period longer than three days or repeated treatment might have reduced the number of the carriers. Furthermore, the immune system was relatively immature during the period of the experiment. These findings are in agreement with those of Newnham (1963), Jordan *et al.* (1978), Jordan and Knight (1984), Migaki *et al.* (1993) and Tanner *et al.* (1993).

**REFERENCES**


عقار الدانوفلوكساسين كمضاد قوي للميكوبلازما

مثال للأكرام محمد

معهد بحوث صحة الجهاز - مركز البيولوجيا المlekية - نقل - جنوب - مصر.

تم اختيار كعكة الدانوفلوكساسين في علاج ووقاية كتاشكفيت المتعدد المجرى
التجهيزية، ولهذا الغرض تم استخدام مادة كتاشكفيت من نوع هيبرد وتم تقديم هذه
الكتاشكفيت في خمس مجموعات متساوية بعد 10 يوم من بداية التجربة.

تتم عن طريق العمود الأول بعشرة啐رة خارجية للميكوبلازما والسيكيم من نوع اسم 1 - 10
والتي تم تنفيذ عقار الدانوفلوكساسين بعد 50 جرعة في الملون وذلك بعد يوم واحد من
ظهور الامراض، والمجموعة الثالثة تم استخدامها نفس الجرعة لمدة ثلاثة أيام ككفاءة تم بعدها
إجراء الاختبار الشحمي والمعدة بعشرة الميكوبلازما التاسع 10 - 10 وتم بعدها العلاج باريغ
وامتحان ساعة من ظهور الامراض، والمجموعة الثالثة تم استخدامها عقار الدانوفلوكساسين
دون اجراء العلاج وذلك لمدة سبعية أيام وكانت هذه ميزة مجموعلا القتاليات، والمجموعة
الرائعة كانت ميزة ضعيفة نسبياً بمعنى عدم العلاج أو العدوى التجهيزية والمجموعة
الخاصة تم اعتبارها ميزة الضجيج الإيجابي بمعنى العدوى مع عدم العلاج.

وقد أظهرت النتائج أن الجرائم التي أعطيت جرعة واحدة من الدواء تم أجريت
عليها العدوى التجهيزية ظهر فيها زيادة وزن الجسم وكذلك وزن الأعضاء الداخلية
بالنسبة لغيرها من المجموعات. مما هو جدير بالذكر أن الأعراض الإكلينيكية كانت شديدة
في المجموع الذي كانت ميزة الضجيج الإيجابي وكذلك الدواوين التي تمت فيها العدوى
التجهيزية وأعطيت عقار الدانوفلوكساسين بعد 24 ساعة من ظهور الامراض وقد كانت
نسبة الفشل 40% على الشكل في المجموعتين. وقد تم قبول الميكوبلازما
والسيكيم في الدواوين التي تم فيها العدوى ثم أعطيت الدواء وكذلك الكتاشكفيت التي تمت
العديد التجهيزية وأعطيت العدوى الوقائية وكذلك الدواوين التي أعطيت كتاشكفيت
الإيجابي.

أما بالنسبة لعدو ثلاثان الدم الإكليني فقد كان عاديًا في المجموعة الأولى وذلك
خلال الأسبوع الأول بعد إعطاء الدواء ثم انخفض العدوي في الأسبوع الثاني بينما في
المجموعة الثانية كان انخفاضًا خلال الأسبوع الأول والثاني بعد إعطاء الدواء، أما في
جميع مجموعات الإكليني والضجيج السهلي فقد كان معدل الثلاثان منخفضا في الأسبوع
الثاني.