Pathological Studies on Danofloxacin and Tilmicosin used for Treatment of Chronic respiratory Diseases in chicks

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ABSTRACT

Seventy of commercial broiler chicks of 2 week old ages were used to study the efficiency of either or both Danofloxacin and Tilmicosin in experimental chronic respiratory diseases (CRD) infection. The latter was induced by simultaneous infection of both mycoplasma gallisepticum with E.coli. Experimental groups divided into 7 groups (Gps) of 10 chicks. Gp. 1 was left without treatment, gp. 2 was orally given 2 ml distilled water, gp. 3 received 0.1ml/kg B.wt danofloxacin, gp. 4 received 1.2 ml/kg B.wt from tilmicosin and gp. 5 were inoculated with 0.2 ml inoculum of both mycoplasma gallisepticum (MG) and E.coli (O78) intra-tracheally route. Meanwhile Gp .6 and 7 were the experimental groups where the infected chicks received 0.1ml/kg B.wt danofloxacin and 1.2 from tilmicosin orally for five consecutive days after the clinical signs appeared by 24 h respectively. Necropsy was performed weekly; clinical signs and gross lesions were recorded. Specimens from liver, heart, lungs, kidneys, air sac, brain, spleen, bursa of fabricius were collected from all the necropsied chicks. In gp.5, the main clinical signs were severe depression, anorexia and respiratory signs, while macroscopically were septicemic lesions were seen 1 and 2 weeks post inoculation (PI) together with fibrinous perihepatitis, pericarditis and airsaculitis. Microscopically, the liver showed several areas of central cavitations surrounded with a zone of coagulative necrosis with perihepatitis represented by thickening in the hepatic capsule by fibrinous exudates and leukocytes infiltrations. The heart showed thickened pericardium with serofibrinous exudate entrapping macrophages, lymphocytes and few heterophils. The trachea showed focal areas of necrosis or ulceration with extensive replacement of the mucosa with leukocytes. The lungs revealed more serious lesions which represented by severe bronchitis and focal pneumonia. The air sacs showed heterophils and mononuclear cells infiltrations, particularly at the first 2 weeks. The spleen showed necrosis and depletion of lymphocytes from white pulp. Meanwhile in gp 6, the danofloxacin were alleviated the severity of the lesions and in gp. 7 tilmicosin complete absent of the lesions.

INTRODUCTION

Infectious diseases are the real threat to poultry industry. Chronic Respiratory Disease (CRD) is of prime importance which caused by E. coli and Mycoplasma gallisepticum (1). Mycoplasma gallisepticum can cause significant economic losses on poultry farms where it decreased growth rate and decreased egg production (2).

There is no doubt that the availability of other pathogens such as Escherichia coli and environmental stress factors play important roles in MG infection (3).

Escherichia coli spread into various internal organs and cause colibacillosis (4). Colibacillosis is one of the main causes of economic losses in the poultry industry worldwide, from mortality, decrease in
productivity of the affected bird, retard growth and condemnation at slaughter houses (5).

Danofloxacin and Tilmicosin are antimicrobial drugs used for prevention and control of CRD (6). Danofloxacin is a synthetic antibiotic of the fluoroquinolone group which has bacteriocidal activity against most Gram-negative bacteria, some Gram-positive bacteria and mycoplasmas (7).

Tilmicosin is a broad-spectrum bacteriostatic macrolide antibiotic synthesized from tylosin against Mycoplasma spp., Pasteurella spp. and various Gram-positive organisms (8).

MATERIAL AND METHODS

Experimental designs

Seventy commerical broiler chicks of 2 week old age free from mycoplasma or bacterial infection were divided into 7 groups (Gps) of 10 chicks. They were housed on floored battery cages under hygienic condition. Water and commercial starter ration were provided with ad libitum. Gp. 1 was left without treatment, gp. 2 was orally given 2 ml distilled water, gp. 3 received 0.1 ml/kg B.wt danofloxacin, gp. 4 received 1.2 from tilmicosin and gp. 5 were inoculated with 0.2 ml inoculum of both mycoplasma gallisepticum (MG) and E.coli intra-tracheally route. Meanwhile Gp .6 and 7 were the experimental groups where the infected chicks received 0.1 ml/kg B.wt danofloxacin and 1.2 from tilmicosin orally for five consecutive days after the clinical signs appeared by 24 h respectively.

The MG (field strain) which used at concentration of approximately 1x10^9 CFU/ml (9) and E.coli O78 at concentration of approximately 1x 10^9 CFU/ml (10) were obtained from department of bacteriology, faculty of vet. Medicine, Zagazig University.

Danofloxacin manufactured by Marcyrl pharmaceutical industries El Obour city while Tilmicosin manufactured by ChemVet.

Clinical signs and Post Mortem examination

The chicks were observed daily for any respiratory signs or dead cases. A chick from each group was randomly selected weekly at the end of 1st, 2nd, 3rd, 4th, 5th and 6th week PI, slaughtered and examined macroscopically for any lesions in the internal organs.

Histopathological examination

Specimens from from liver, heart, lungs, kidneys, air sac, brain, spleen, bursa were collected from all the necropsies chicks and fixed in 10% buffered neutral formalin solution. Five-micron thick sections are prepared and stained with hematoxylin and eosin stain (HE) then examined microscopically (11).

RESULTS

Gp.1, 2, 3, 4 showed neither gross nor microscopic lesions.

Gp.5, the chicks were inoculated with both mycoplasma gallisepticum (MG) and E.coli showed severe depression, anorexia, ruffled feather, coughing, respiratory rales, sneezing and rapid mouth breathing, within the first 3 weeks PI. Loss of weight, poor growth and emaciation were seen, 4 and 6 weeks PI. Macroscopically, septicemic lesions were observed after 1 and 2 weeks PI. Fibrinous perihepatitis, pericarditis and airsacculitis were also detected.

Microscopically , the liver showed several areas of central cavitations containing numerous heterophils and surrounded with a zone of coagulative necrosis infiltrated with leukocytes, 1 and 2 weeks PI (Figs 1 and 2). Perihepatitis represented by thickening in the hepatic capsule by fibrinous exudates and leukocytes infiltrations was noticed, 1 and 2 weeks PI. Such exudates were focally
organized into fibrous connective tissue and infiltrated with macrophages and lymphocytes, later on (Fig 3). The heart revealed thickened pericardium with serofibrinous exudate entrapping macrophages, lymphocytes and few heterophils, 1 and 2 weeks PI. Such exudate became organized later on and gradually replaced by granulation tissue, infiltrated with macrophages, giant cells of foreign body type and lymphocytes, 3 - 6 weeks PI (Fig 4). The trachea showed focal areas of necrosis or ulceration with extensive replacement of the mucosa with leukocytes of mostly lymphocytes, heterophils and macrophages, in the first 2 weeks PI (Figs 5 and 6). The others showed hyperplasia in the lining epithelium with increased number of goblet cells together with congested blood vessels, edema in the submucosa and leukocytic infiltrations, in chicks which were sacrificed from 3rd to 6th weeks PI. The lungs revealed more serious lesions which represented by severe bronchitis and focal pneumonia. The bronchi showed severe thickening in the mucosa with epithelial hyperplasia, metaplasia into goblet cells and desquamation besides leukocytes infiltrations, congested capillaries and edema, 1 and 2 weeks PI (Figs 7 and 8). Extensive fibrous connective tissue proliferation infiltrated with mononuclear cells was detected around the affected bronchi, from 3 to 6 weeks PI (Fig 9). While, the pneumonia became intense and involving several pulmonary lobules and contained mixed aggregations of heterophils and lymphocytes in the first 2 weeks PI and aggregations of plasma cells, macrophages and lymphocytes, in 3 and 4 weeks PI. These cells were mostly macrophages and few lymphocytes, 5 and 6 weeks (Fig 10). The air sacs in this group were extensively involved and showed heterophils and mononuclear cells infiltrations, particularly at the first 2 weeks PI. Areas of necrosis in air sacs were often surrounded with macrophages, lymphocytes and giant cells besides fibroblasts proliferation were seen later on (Fig 11). The spleen showed necrosis and depletion of lymphocytes in the white pulp (Figs 12).

Gp. 6, the infected chicks were received danofloxacin showed ruffled feathers and slightly anorexia particularly at the first week post treatment (PT). Meanwhile the birds which were sacrificed later were apparently healthy with normal activities and performance. Macroscopically, were slightly alleviated in the severity of the lesions than those described in infected group where the sacrificed bird showed slightly congestion of liver, lungs and trachea. The heart showed grayish white foci (1-2 mm in diameter) during the first week PT. Microscopically, The liver showed congestion of hepatic blood vessels with few lymphocytes aggregations in the portal areas and mild hyperplasia in the lining epithelium of bile ducts (Fig 13). The heart showed congested blood vessels with thickened and hyalinized in its wall. The pericardium was slightly thickened with edema and few lymphocytes infiltrations. The trachea showed mild hyperplasia, mucinous degeneration and focal desquamation of the lining epithelium with edema and mononuclear cells infiltrations in the submucosa. The lungs showed moderate congestion of pulmonary blood vessels. Some bronchi were lined by hyperplastic epithelium with mucinous degeneration and their wall was infiltrated with round cells (Fig 14). The air sacs of few birds revealed congested blood vessels and few leukocytic infiltrations of predominately lymphocytes. The spleen revealed mild depletion of lymphocytes inside white pulps only at the first week PT, later on, the white pulp were restored to the normal architectures.

Gp. 7, the infected chicks were received tilimicosin, The lesions which described in the previous groups were completely absent from the examined organs which appeared normal except in some cases showed few round cells infiltrations in the portal areas of the liver. Focal hydropic degeneration and vacuolation in the hepatocytes (Fig 15). The lungs revealed slight congestion in the pulmonary blood vessels. The spleen showed normal lymphoid follicles or mild hyperplasia in the lymphocytes of white pulp. Some splenic blood vessels showed thick and hyalinized wall (Fig 16). The remaining organs were normal or with slight congestion of its blood vessels.
Fig 1. Photomicrograph of liver, gp (5) 7 day PI, showing area with central cavitations containing numerous heterophils and lymphocytes (arrow) surrounded by a zone of coagulative necrosis infiltrated with leukocytes (arrow head), HE x300.

Fig 2. A high magnification of previous fig (1) to show the coagulative necrosis (arrow) surrounding the cavitations, HE x1200.
Fig. 3. Photomicrograph of liver, gp (5) 21 day PI, showing perihepatitis with thickening the hepatic capsule with fibroblast proliferation and round cells infiltration (arrow), HE x300.

Fig. 4. Photomicrograph of heart, gp (5) 14 day PI, showing pericarditis represented by serofibrinous exudate with round cells infiltration and fibroblast proliferation (arrow), HE x300.
Fig. 5. Photomicrograph of trachea, gp (5) 7 day PI, showing partial desquamation with focal mucosal lymphoid aggregation (arrow) and edema in the submucosa (arrow head), HE x300.

Fig. 6. A high magnification of the previous fig (5) to show the necrotic mucosa and round cells aggregations (arrow) besides the edema in the submucosa (arrow head), HE x1200.
Fig. 7. Photomicrograph of lung, gp (5) 7 day PI, showing catarrhal bronchitis with hyperplasia and metaplasia in the lining epithelium (arrow) and round cells infiltrations (arrow head), HE x300.

Fig. 8. A high magnification to show the catarrhal bronchitis with hyperplasia and metaplasia in the lining epithelium (arrow head) and round cells infiltrations (arrow), HE x1200.
Fig. 9. Photomicrograph of lung, gp (5) 21 day PI, showing extensive fibrosis (arrow head) and mononuclear cells infiltration around the inflamed bronchus (arrow), HE x1200.

Fig. 10. Photomicrograph of lung, gp (5) 7 day PI, showing perivascular aggregation of round cells and few fibroblasts proliferation (arrow), HE x1200.
Fig. 11. Photomicrograph of Air sac, gp (5) 14 days PI, showing area of necrosis (arrow) surrounded with macrophages, lymphocytes and few heterophils besides fibroblasts proliferation, HE x1200.

Fig. 12. Photomicrograph of spleen, gp (5) 7 day PI, showing extensive necrosis and depletion in the lymphocytes of white pulp, HE x300.
Fig. 13. Photomicrograph of liver, gp (6) 1 week PT, showing portal area with aggregation of few lymphocytes (arrow) and mild hyperplasia in the biliary epithelia (arrow head), HE x 1200.

Fig. 14. Photomicrograph of lung, gp (6) 7 day PT, showing bronchi lined by hyperplastic epithelium with mucinous degeneration (arrow) and round cells infiltration (arrow head), HE x 1200.
Fig. 15. Photomicrograph of liver, gp (7) 7 day PT, showing mild hydropic degeneration and vacuolation in the hepatocytes, HE x 1200.

Fig. 16. Photomicrograph of Spleen, gp (7) 7 day PT, showing mild hyperplasia in the lymphocytes of white pulp (arrow) and thickening in the wall of splenic artery (arrow head), HE x 1200.
Moustafa et al.,

DISCUSSION

Of great importance is the ability of E.coli to act synergistically with mycoplasma gallisepticum leading to CRD in broiler chicken. These study aim to study the pathological lesions induced by experimental colibacillosis and mycoplasmosis and to investigate the efficiency of Danofloxacin and Tilmicosin against these lesions.

The infected chicks of (gp 5) showed severe depression, anorexia, ruffled feather, coughing, respiratory rales, sneezing and rapid mouth breathing, within the first 3 weeks PI. Loss of weight, poor growth and emaciation were seen, 4 and 6 weeks PI. The post mortem lesions were septicemic lesions, 1 and 2 weeks PI. Fibrinous pericarditis, pericarditis and airsaculitis were also detected. Similar findings were recorded previously (12). Microscopically, the liver showed several areas of central cavitations surrounded with a zone of coagulative necrosis infiltrated with leukocytes, 1 and 2 weeks PI. Pericarditis represented by thickening in the hepatic capsule by fibrinous exudates and leukocytes infiltrations was noticed, 1 and 2 weeks PI. The heart of all chicks showed thickened pericardium with serofibrinous exudate entrapping macrophages, lymphocytes and few heterophils, 1 and 2 weeks PI. The trachea showed focal areas of necrosis or ulceration with extensive replacement the mucosa with leukocytes of mostly lymphocytes, heterophils and macrophages, in the first 2 weeks PI. The lungs revealed more serious lesions which represented by severe bronchitis and focal pneumonia, 1 and 2 weeks PI. Similar findings were recorded in mycoplasma and ecoli infection (13). The airsacs were extensively involved and showed heterophils and mononuclear cells infiltrations, particularly at the first 2 weeks PI. Similar findings were recorded in mycoplasma gallisepticum in poultry (14). The spleen showed necrosis and depletion of lymphocytes from the white pulp and lymphoid follicles.

Generally the histopathological lesions of CRD due to mycoplasma which can cross the respiratory membrane barrier by their virulence factor to enter blood stream induce systemic lesions with E.coli in various internal organs. Our results were also described by (15).

The chicks in Gp.6 showed ruffled feathers and slightly anorexia particularly at the first week PT. Meanwhile the birds which were sacrificed later were health with normal activities and performance. Macroscopical lesions were slightly alleviated in the severity than those described in infected group. The sacrificed bird showed slightly congestion of liver, lungs and trachea. The heart showed greyish white foci (1-2 mm in diameter) during the first week PT. microscopically, the liver showed congestion of hepatic blood vessels with few lymphocytes aggregations were detected in the portal areas. The heart showed congested blood vessels with thickened and hyalinized in their wall. The pericardium was slightly thickened with edema and few lymphocytes infiltrations. The trachea showed mild hyperplasia, mucinous degeneration and focal desquamation of the lining epithelium with edema and mononuclear cells infiltrations in the submucosa. The lungs showed moderate congestion of pulmonary blood vessels. Some bronchi were lined by hyperplastic epithelium with mucinous degeneration and their wall was infiltrated with round cells. The airsacs of few birds revealed congested blood vessels and few leukocytic infiltrations of predominately lymphocytes. The spleen revealed mild depletion of lymphocytes inside white pulp only at the first week PT; meanwhile in the latter, the lymphoid follicles and white pulp were restored to the normal architectures. Similar findings were recorded after the use of danofloxacin in broiler chicks (16).

The chicks in Gp.7 showed complete absent of the lesions that described in previous groups except in some cases showed few round cells infiltrations in the portal areas of the liver with focal hydropic degeneration and vacuolation in the hepatocytes. The lungs revealed slight congestion in the pulmonary blood vessels. The spleen showed normal lymphoid follicles or mild hyperplasia in the
lymphocytes of white pulp. Some splenic blood vessels showed thick and hyalinized wall. The remaining organs were normal or with slight congestion of its blood vessels. Similar pathological findings were recorded in broiler chicks treated with tilmicosin (17).

Both drugs improvement the lesions of CRD as accumulate in high concentrations in the mucosal membranes of respiratory and genitourinary tract the aforementioned findings described by tilmicosin in control mycoplasma gallisepticum (18).

In conclusion, the findings of the present work indicated that MG and E.coli together could induce CRD lesions. Both danofloxacin and tilmicosin improved the lesions of CRD but tilmicosin induced complete absent of the lesions.

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المملوكة العربي

دراسات باثولوجية على بعض المضادات الحيوية المستخدمة في علاج الأمراض المزمنة

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هذه الدراسة تعطي الوضوء على أهم الأفلاط المرضية الناجمة عن داء الميكروب القولوني وداء المفتورات (اليمكولازما جاليسبنكم) تجريبيا للتحقيق أيضا في مثبط كلا ملد دواء دانوفلوكساسين وتموكوزين ضد هذه الأفلاط إذا تم استخدام حوالي سبعون كنتوكت تممهم عمر سبوعين وتم تقسيمهم إلى سبع مجموعات (عشرة كنتوكت لكل مجموعة). لهذه الدراسة

المجموعة الأولى تم تركها بدون علاج أما المجموعة الثانية تم إعطائها 3 مل من الماء المنظف عن طريق الفم. المجموعة الثالثة تم إعطائها دواء دانوفلوكساسين جرعة 0.1 مل لكل كيلو جرام من وزن الطائر والمجموعة الرابعة تم إعطائها دواء تلميكوزين جرعة 0.2 مل لكل كيلو جرام من وزن الطائر عن طريق الفم أما المجموعة الخامسة تم إعطائها كلا من دواء الميكروب القولوني ونينيكوزين (الميكولازما جاليسبنكم) 0.2 مل داخل القصبة الهوائية. بينما المجموعة السادسة والإستثناء هي التجربة العملية حيث أن الكنوكات المعدى تم علاجه بدواء دانوفلوكساسين جرعة 0.1 مل لكل كيلو جرام من وزن الطائر ويدوء
وبداء تليميوزين جرعة 1.2 مل لكل كيلو جرام من وزن الطائر عن طريق الفم لمدة 5 أيام متتالية على التوالى. بعد ظهور الأعراض ب 24 ساعة. تم تجميع العينة من الكبد، القلب، الرئتين، الحويصلات الهوائية، المنًا - الطحال، الأمعاء الدقيقة والغليظة) اسبوعيا بعد اجراء صحة التشريح المرضي للكشف عن أي افلاس مرضية. وتم تسجيل الاعراض الاكلينكية والتغيرات المجهرية.

أهم الاعراض الاكلينكية في المجموعة الخامسة هي الخمول وفقدان الشهية، اعراض تنفسية. أما أهم التغيرات العينية هي التسمم الحشوي وظهور في الأسبوع الأول والثاني من العدوى مع وجود الالتهاب القبلي لجدار الكبد والتاميور والتهاب الحويصلات الهوائية وفحص الكبد وفحص عدة مناطق من التجاوف المركزية محاطة بمنطقة النخر مع التهاب حوائط الكبد مثيلة لسماكة في كبسولة الكبد عن طريق الإفرازات القبلي والكريات البيضاء كما أظهر القلب سماكة في التاميور عن طريق الإفرازات الفيبرينية المختلطة مع بعض الأفرازات المصلية مع وجود الخلايا الليمفاوية وقليل من الخلايا البيضاء المعدة. وأظهرت القصبة الهوائية منطقة من النخر أو التقرح مع استبدال واسع للغشاء المخاطي بالكريات البيضاء. واظهرت الرئتين أكثر الافرازات خطورة المتمثلة في التهاب الشعب الهوائية والالتهاب الرئوي الحاد. كما أظهرت الحويصلات الهوائية تسلي الخلايا وحيدة النواة وخلايا البيضاء المعدة وخاصة في بداية الأسبوع الثاني. وفحص الطحال لوحظ نخر واستنزف الخلايا الليمفاوية من البصيلات المفاوية. بينما أظهرت في المجموعة السادسة ان دانوفلوكسيسون له تأثير مؤثر حيث ان ظهور تخفيف من عدة الأفلاس واما المجموعة السابعة.

فقط أظهر دواء تليميوزين غياب كامل في الافات.

في النهاية نلاحظ في هذه الدراسة ان دواء الميكروب القولوني وداء المفطورات (الميكوبلازما جاليسيتكم) مما احدث افلاسات المرض التنفسي المزمن. وكلا من دواء دانوفلوكسيسون ادواء تليميوزين اظهروا تحسن في علاج المرض التنفسي المزمن ولكن تليميوزين اظهر غياب كامل في الافات.