Comparative studies on pulse and continuous oral norfloxacin treatment in broilers and turkeys

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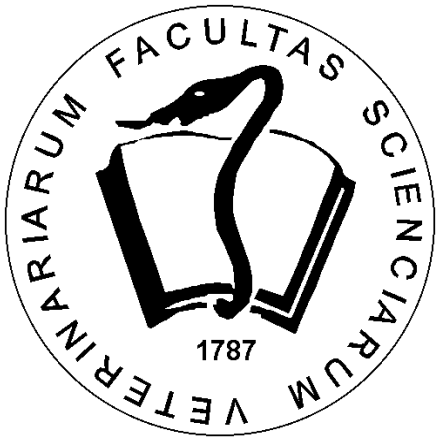

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I. INTRODUCTION

The fluoroquinolones are the most promising molecules of the last decade and many articles and books were published since 1990 about their properties.

Over the last two decades, research on 4-quinolone-3-carboxylates has led to the discovery of a family of 6-fluoro-7-piperazinyl-4-quinolones active against Gram-negative and Gram-positive bacteria in vitro as well as intracellular pathogens and trimethoprim/sulfonamide resistant microbes; in addition these antimicrobials are also active against mycoplasmas. Collectively, these compounds are called fluoroquinolones. Although dozens of fluoroquinolones have been synthesized and reported, the most notable ones being developed, or used, in veterinary medicine worldwide include (in alphabetical order) amifloxacin, benofloxacin, ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, marbofloxacin, norfloxacin and norfloxacin nicotinate, ofloxacin, orbifloxacin and sarafloxacin. Enrofloxacin was the first fluoroquinolone introduced into veterinary medicine. All fluoroquinolones are bactericidal and all act on the same bacterial target: the bacterial DNA gyrase (type II topoisomerase). No plasmidic resistance against them has been demonstrated. Resistant bacteria show cross reactivity for the different quinolones and fluoroquinolones but no

cross reactivity with other antimicrobial families.

These fluoroquinolones share a great oral bioavailability in all monogastric species, a large volume of distribution and a low binding to plasma proteins that allows them to cross membranes and reach the most remote parts of the body at concentrations above the minimum inhibitory concentrations (MIC's) of most pathogens. Tissues and sites demonstrating high concentrations following systemic administration include the kidney, liver and bile plus the prostate, female genital tract, bone and inflammatory fluids. They are eliminated for the most part in the urine and reach levels 100 to 300 times more concentrated in the urine than in the serum.

Norfloxacin is a third generation fluoroquinolone that was first introduced for treating urinary tract infections in humans. Later it became popular in the veterinary medicine as well and so it was approved for use in poultry in 1990. The most attractive characteristics of norfloxacin are good absorption when given orally, and maintenance of effective serum and tissue levels against a broad range of pathogens causing systemic infections.

Post-antibiotic effects (decreased or abnormal growth of bacteria after exposure to an antibacterial agent: PAE) lasting 4-8 hours have been seen in a number of strains after the administration of the fluoroquinolones. The PAE is associated with decreased adherence to cells as part of the phenomenon. Concentrations as low as 1000 fold less

than the MIC have been shown to decrease adherence of *Staphylococcus aureus* bacteria to buccal cells even though the PAE is concentration dependent.

Current expectations are that C_{max} (maximal plasma concentration) will be more closely related to reducing resistance and a 2-3 fold C_{max}/MIC (minimum inhibitory concentration) ratio is sufficient for optimal antimicrobial action. A pulse-dose regimen of fluoroquinolones has been suggested and widely used with the aim of achieving high peak concentrations and exploiting the post-antimicrobial effect.

Colibacillosis, caused by *Escherichia coli* (*E. coli*), is a bacterial infection in chickens and turkeys may result in septicaemia, respiratory tract infections, pericarditis, peritonitis and airsacculitis. *E. coli* may also be associated with other agents, such as infectious bronchitis virus (IBV), Newcastle disease virus (NDV) including vaccine strains, *Mycoplasma spp*., *Pasteurella spp*., causing the respiratory disease complex. Although this disease is related with a number of pathogens, infection with *E. coli* is of particular concern because it commonly progresses to a more generalized condition associated with high mortality and condemnation losses at processing.

Avian cholera (fowl cholera) caused by the Gram-negative, nonmotile, coccobacillary bacterium *Pasteurella multocida*, has been known to occur in a variety of wild and domestic birds and cause major economic losses in the poultry industry. Death results primarily from

peracute and acute septicemia and less frequently from the chronic and localized form of the disease. Most reported outbreaks of fowl cholera affected chickens, turkeys, ducks and geese but *Pasteurella multocida* has also been known to cause devastating outbreaks in free-living waterfowl. The wide range of avian hosts in which fowl cholera has been reported suggests that all species of birds are susceptible. The most sensitive are young mature turkeys; but all ages are highly susceptible.

The lack of information on the pharmacokinetics of continuous- and pulse dosing treatment and the unproven hypotheses in the veterinary situation that he AUIC is closely related to efficacy, together with the high importance of the poultry pathogen *Escherichia coli* and *Pasteurella multocida* infections, led us to compare the pharmacokinetic properties and efficacy of pulse dosing oral norfloxacin treatment with that of an established medication of continuous dosing, in broiler chickens and turkeys.

II. AIMS OF THE PRESENT STUDY

The principal application of norfloxacin has been for gastrointestinal and respiratory infections. While pharmacokinetic evaluation of norfloxacin has been carried out in normal chickens, after extensive literature searches there is a lack of information on the clinical use of norfloxacin in chickens and turkeys in the treatment of fowl cholera and *Escherichia coli* infection and its pharmacokinetic and pharmacodynamic interrelationship during disease. We planned to develop an insight into the pharmacodynamics of outcome for the treatment of infections with the fluoroquinolones.

The basic questions that have been studied are:

- Implementation and development of a *Pasteurella multocida* and *Escherichia coli* infection model that is steadily functional for evaluating the efficacy of antimicrobial medication.

- Characterization of the efficacy of pulse- and continuous

dosing oral norfloxacin treatment in the control of induced pasteurellosis and colibacillosis in broiler chickens and turkeys.

- Obtaining information about the general pharmacokinetic features of norfloxacin to determine the properties of pulse- and continuous oral administration in chickens and turkeys.

- Investigation of pharmacodynamics to establish optimal therapeutic dose and dose interval as well as determine the usefulness of pulse and continuous oral administration in chickens and turkeys. Optimizing one or both of these ratios may ultimately reduce the likelihood that microbial flora will develop resistance.

III. CONCLUDING REMARKS

As with many other agents, there is considerable confusion over the most appropriate way to administer this drug in order to obtain optimal outcome for both high and/or low pathogenicity infections in poultry.

A regimen of large doses (resulting in high AUC and C_{max}) given

infrequent intervals, such as pulse dosing (thus relying on the PAE) has been suggested to be more efficacious in terms of bacterial killing, eradication time, and reducing the selection of resistant bacteria. This suggestion, together with the importance of *Pasteurella multocida* and *Escherichia coli* as a poultry pathogen, led us to compare the pharmacokinetic properties and efficacy of pulse dosing oral norfloxacin treatment with that of an established medication of continuous dosing in broiler chickens and turkeys.

The efficacy of artificial infection model relies on the virulence and the challenge dose of utilized bacteria. In our studies we were able to successfully develop a reliable method for both *Pasteurella multocida* and *Escherichia coli* infection that made possible to answer the basic questions.

Treatment of Pasteurella multocida infection

Norfloxacin administered at 100 mg/L, continuous dosing was as efficacious as norfloxacin administered at 15 mg/kg pulse dosing in chickens. There was no difference in daily weight gain, slight variation in mortality, but well characterized alteration in postmortem scores, daily clinical scores and recovery of bacteria. It should be considered that the inoculum was approximately 80 CFU X-73 (A:1), close to its LD_{50} (19.6 CFU) value. In turkeys, where 15 fold (approximately 70 CFU P-1059 (A:3)) of the LD_{50} (4.67 CFU) was used, significant alteration in all

examined parameters was observed, except average daily weight gain, between the two different treatment schedule. We can proclaim that continuous dosing in chickens and pulse dosing in turkeys were significantly more valuable in treating *Pasteurella multocida* infection in the present study. These results were exactly the opposite as was expected by the pharmacokinetic properties described in the literature.

Some literature review indicated that even with an 8 hour lasting PAE the fluoroquinolone treatment gives better result using as pulse-dose medication in drinking water, while others showed that severe bacterial infections are better treated with high dosages of antimicrobials. These results confirm our findings that low volume bacterial infection, 4 fold over the LD_{50}, improves with continuous dosing, while severe bacterial infection, 15 fold over the LD_{50}, advances better following a pulse dosing schedule.

Treatment of Escherichia coli infection

The *E. coli* model used in the chick-inoculation experiment produced 40% mortality and 1.77±1.27 daily clinical score (60% morbidity) in infected, non-medicated birds. These levels compare favorably to the 5% mortality and up to 50% morbidity suggested by different authors as being typical for colibacillosis in the field. In the present study, both treatment procedures effectively reduced the experimental colibacillosis in chickens. Norfloxacin administered at 15 mg/kg pulse dosing was

more efficacious then norfloxacin administered at 100 mg/L, continuous dosing in chickens. There was no variation in daily weight gain and mortality, but well characterized alteration in daily clinical scores, postmortem scores for the benefit of pulse dosing. The results obtained in this study coincide with the conclusion from the pharmacokinetic properties described in the literature.

These differences can be explained if we assume that low-level bacterial infection gives better recovery with continuous dosing, while severe bacterial infection shows better improvement following pulse-dosing schedule.

Pharmacokinetic properties

The plasma norfloxacin concentrations increased slowly during continuous dosing and reached the MIC for the most Gram-negative pathogen bacteria by 12 h in chickens and 18 h in turkeys. The mean steady-state plasma concentration was also attained in 36 h. It remained approximately at the same plasma concentration level both in chickens and turkeys (776.67±33.23 ng/ml in chickens and 682.50±28.55 ng/ml in turkeys) during the whole treatment period. Pulse dosing produced half the steady-state concentration (365.32±39.31 ng/ml in chickens and 306.03±32.26 ng/ml in turkeys) as it was obtained after continuous dosing.

After pulse dosing the plasma norfloxacin concentrations

increased rapidly and significantly exceeded the MIC at 2 h in both chickens and turkeys and remained above for 8 h in chickens and 6 h in turkeys. Data of the daily pulse dosing suggested that every administration of the drug corresponds to a single, daily repeated bolus administration. However pulse dosing achieved higher plasma concentration more readily then continuous dosing.

Fluoroquinolones show concentration-dependent killing *in vitro*, and animal models have demonstrated that the principal predictors of *in vivo* killing are the 24-hour AUIC (AUC/MIC) and the C_{max}/MIC ratio. The AUIC appears to be important for killing, whereas the C_{max}/MIC ratio is important to prevent the selection of resistance mutants during treatment. During *in vivo* studies, bacteriostasis was achieved with an AUIC of around 35, while in a variety of animal models mortality is completely prevented once the ratio reached 100.

However, prevailing ideas are that C_{max}, over 2-3 times the MIC, is more closely related to reducing resistance. In our studies the C_{max} exceeded the MIC over 5-10 times for the strains used and the AUIC was over 100, however substantial differences were obtained between the dosing schedules. Our findings support the view that low pathogenicity bacterial infection gives better recovery with continuous

dosing, while severe bacterial infection shows improving effect following pulse dosing schedule.

It can be concluded that regimens of continuous dosing at 100 mg/L or pulse dosing medication at 15 mg/kg may be used when administering norfloxacin in herd situations, however, we recommend to treat bacterial infections of either high or low pathogenicity starting with pulse dosing for 4 hours and then maintaining continuous oral medication for 3-5 consecutive days. Nevertheless manufacturers recommend using fluoroquinolones such as norfloxacin in a pulse-dosing manner throughout the whole treatment period.

Our recommended method of administration should help in preventing both the severe and less serious bacterial infections and could prevent the emergence of resistance against the fluoroquinolones.

Fluoroquinolones are one of the most useful classes of antimicrobial agents used in human and animal medicine today, both because of their spectrum and their physicochemical properties. As such, their popularity in clinical situations is increasing.

Recently, however, concerns have been raised over the possible emergence of quinolone-resistant strains and the

effects on the environment if such drugs are overused. At present it appears that, physicians and veterinarians can prolong their usefulness for many years if they use appropriate clinical judgment and proper dosing principles as they prescribe and administer these drugs to patients.

If used in a well-controlled manner, quinolones will contribute greatly to stock farming management, without adversely influencing human chemotherapy.

IV. LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers:

SÁRKÖZY G. (2002) QUINOLONES: A CLASS OF ANTIMICROBIAL AGENTS. *VETERINARY MEDICINE – CZECH*, **46** (9-10): 257-274.

SÁRKÖZY G., SEMJÉN G., LACZAY P. AND HORVÁTH E. (2002) TREATMENT OF EXPERIMENTALLY INDUCED *PASTEURELLA MULTOCIDA* INFECTIONS IN BROILERS AND TURKEYS - COMPARATIVE STUDIES ON DIFFERENT ORAL TREATMENT REGIMENS. *JOURNAL OF VETERINARY MEDICINE SERIES B*. **49**: 130-134.

SÁRKÖZY G., SEMJÉN G., LACZAY P., HORVÁTH E. AND SCHMIDT J. (2002) PULSE AND CONTINUOUS ORAL NORFLOXACIN TREATMENT OF EXPERIMENTALLY INDUCED ESCHERICHIA COLI INFECTION IN BROILER CHICKS AND TURKEY POULTS. *ACTA VETERINARIA HUNGARICA*, 2002, **153**: 199-210.

SÁRKÖZY G., SEMJÉN G. AND LACZAY P. 2002. PHARMACOKINETIC COMPARATIVE STUDIES ON DIFFERENT ORAL TREATMENT REGIMEN IN BROILERS AND TURKEYS. *JOURNAL OF VETERINARY PHARMACOLOGY AND TOXICOLOGY*, (PRESENTED FOR PUBLICATION).

V. OTHER SCIENTIFIC PUBLICATIONS

Posters:

SÁRKÖZY, G. AND LACZAY, P. (2001) COMPARATIVE STUDY ON THE EFFICACY OF DIFLOXACIN AND ENROFLOXACIN IN AN EXPERIMENTALLY INDUCED ESCHERICHIA COLI INFECTION IN BROILERS. PROCEEDINGS OF THE 12TH WORLD VETERINARY POULTRY ASSOCIATION CONGRESS, CAIRO, EGYPT. P. 396.

SÁRKÖZY, G. AND LACZAY, P. (2001) COMPARATIVE STUDY ON THE EFFICACY OF DIFLOXACIN AND ENROFLOXACIN IN AN EXPERIMENTALLY INDUCED PASTEURELLA MULTOCIDA INFECTION IN TURKEYS. PROCEEDINGS OF THE 12TH WORLD VETERINARY POULTRY ASSOCIATION CONGRESS, CAIRO, EGYPT. P. 397.