PK-PD modelling of norfloxacin after oral administration in rabbits

B.H. Pavithra*, N. Prakash and K. Jayakumar[1]

Department of Veterinary Pharmacology and Toxicology
Karnataka Veterinary, Animal & Fisheries Sciences University
Veterinary College, Bidar 585 401, Karnataka, INDIA.
1. Department of Pharmacology & Toxicology, Veterinary College, Bangalore-560 024
Corresponding author e-mail: pavithra_bh@yahoo.co.in

Abstract

Norfloxacin posses a wide spectrum of activity, excellent tissue penetration and is rapidly bactericidal at low concentrations and hence an attempt was made to integrate reported pharmacodynamic data with pharmacokinetic data of norfloxacin after oral administration in rabbits to determine its effectiveness against common bacterial pathogens infecting rabbits. Pharmacokinetic data were obtained after a single per oral administration of norfloxacin @ 100mg per kg. Plasma drug concentrations were determined using high performance liquid chromatography (HPLC). From PK-PD integration, it is observed that norfloxacin is highly effective against gram negative infections caused by Pasteurella multocida (AUC/MIC and Cmax/MIC ratio of 133.5 and 111.5 respectively), its efficacy against Salmonella spp., E. Coli, Shigella spp. and Haemophilus influenza is moderate. However, per-oral administration of norfloxacin is not suitable to contain tested gram positive bacterial pathogens infecting rabbits.
Key words: norfloxacin, oral administration, PK-PD integration, rabbits

Introduction

Pharmacokinetic and pharmacodynamic (PK-PD) surrogates have been shown to be important markers for the evaluation of antibiotic regimens and the prediction of the clinical outcome of antimicrobial therapy. The MIC, however, is only one of the factors that need to be considered for the selection of an appropriate antimicrobial agent and dosing regimen (Muller et al., 1996).

Several pharmacokinetic surrogate markers, e.g., peak concentration of drug in serum (Cmax)/MIC ratio; area under the inhibitory curve (area under the concentration-time curve [AUC] /MIC ratio) and time above the MIC (T> MIC) can predict the clinical outcome by a complex combination of pharmaco-kinetic and microbiological parameters (Hyatt et al., 1995).

Norfloxacin (1- ethyl- 6-fluoro-1, 4- dihydro- 4-oxo- 7- (1- piperazinyl) - 3- quinoline carboxylic acid) is a second generation fluoroquinolone having a wide spectrum of activity, excellent tissue penetration and exert bactericidal action at low concentrations, often at a minimum inhibitory concentration (MIC) of less than 0.1 µg.ml-1 (Fernandes, 1988). The individual fluoro-quinolones considerably differ in their physicochemical characteristics, pharmacokinetic properties and therefore CSF penetration (Sorgel et al., 1987). Hence the efficacy predictors like Cop / MIC and AUC0-24/ MIC can predict antimicrobial efficacy of fluoro-quinolones and reduce selection for resistance (Turnidge, 1999).

Keeping these points in view an attempt was made to integrate pharmacodynamic and pharmacokinetic data of norfloxacin so as to determine its effectiveness against common bacterial pathogens infecting rabbits.

Materials and methods

Pharmacokinetic data

The pharmacokinetic data of norfloxacin was derived in adult healthy New Zealand white rabbits. Rabbits (six) were administered with single dose of norfloxacin at the rate of 100 mg/kg per orally. Blood samples were drawn from the marginal ear vein into heparin coated tubes immediately before ('0')and 5, 10, 15, 30, 45 minutes and 1, 1.15, 1.30, 2, 3, 4, 5, 6, 8, 12 and 24 hours after the administration of norfloxacin. Plasma concentration of norfloxacin was determined using High Performance Liquid Chromatography

Table-1. The area under the curve (AUC) and the observed plasma profile of norfloxacin in rabbits after single Oral administration @100mg/kg.

Parameter	Unit	Values*
AUC	µg.h.ml-1	2.67±0.42
Cmax	µg.ml-1	2.23±0.08
Clast detected	µg.ml-1	0.015 ± 0.001
Time at which Clast	h	12

*Values are mean ± S.E

(HPLC; Shimadzu, Japan) as per standard prescribed procedure. The required pharmacokinetic parameters, area under the curve (AUC) and maximum plasma concentration (Cmax) were determined for each experimental animal and the average of these values were used for pharmacokinetic-pharmacodynamic (PK-PD) integration.

Pharmacodynamic data

The MIC50 values of norfloxacin against bacterial species reported by Neu and Labthavikul (1982; Table-1) were utilized for PK-PD integration.

PK-PD integration:

Pharmacokinetic variables derived in the present study was integrated with in vitro pharmaco-dynamic measurements (i.e. established MIC values). The PK-PD analysis was carried out by determining the ratios of (a) area under the inhibitory curve (AUC/MIC=AUIC) and (b) Cmax/MIC according to McKellar et al. (2004) to predict the efficacy of norfloxacin.

Results and discussion

The disposition profile and the pharmacokinetic parameters derived are shown in Table-1.

The computed values of PK-PD integration of norfloxacin is shown in Table-2. The AUC/MIC and Cmax/MIC ratio of 133.5 and 111.5 is very effective in curing serious respiratory tract infection that might be caused by Pasteurella multocida in rabbits, this is because the effective use of fluroquinolones against clinically important animal pathogens is dependant on designing dosing regimens that attain serum AUC:MIC ratios of 125:1 or Cmax/MIC ratios of 10:1(Prescott and Baggot, 2000). Although the AUC/MIC ratio is lower than the recommended value for fluoroquinolnes, the Cmax/MIC ratio of 44.6 is sufficiently high enough to take care of those gram negative organisms like E. coli, Salmonella sp., Shigella sp. which are usually responsible for digestive tract infections. However PK-PD integration of norfloxacin revealed that the either of the two ratios derived (Table-2) fail to kill or arrest the growth of gram-positive organisms like *Staphylococcus aureus , Streptococcus spp.*

The microbiologic activity and pharmacokinetics together define the efficacy of the antibiotic (Nicolau et al., 1995). The dosages and dosing intervals for the common extracellular gram-negative and gram-positive pathogens should be designed with full consideration of the relationships between serum antibiotic concentrations and MICs (Anderson , 1976). In view of difficulties involved, drug concentrations are not measured at the site of activity, a microbiologic parameter such as MIC (minimum inhibitory concentration) or MBC (minimum bactericidal concentration) of the antibiotic is commonly employed as the critical value in the interpretation of these pharmacodynamic relationships. However, pharmacokinetic profile in tissue rather than in serum determines the clinical outcome of antimicrobial therapy. Hence, dosing regimens based on target tissue kinetics would be desirable (Ryan et al., 1986).

For quinolone agents the ratio of Cmax to MIC is considered to be the most relevant surrogate marker and proved to be predictive of bacterial eradication.

Table-2. PK-PD integration of norfloxacin against common bacterial pathogens infecting rabbits.

Pathogen	MIC50*(µg/ml)	PK-PD integration	
		AUIC	Cmax/MIC
Pasteurella multocida	<0.02	133.5	111.5
Salmonella sp.	0.02	133.5	111.5
Shigella sp.	0.05	53.4	44.6
Haemophilus influenzae	<0.05	53.4	44.6
Staphylococcus aureus	0.8	3.34	2.79
Streptococcus pyogenes	1.6	1.67	1.39
Streptococcus faecalis	3.1	0.86	0.71

* Neu and Labthavikul,1982

Although a 99% killing can be obtained by quinolones at a low ratio, e.g., 3 for ciprofloxacin, bacterial regrowth and development of bacterial resistance may occur unless higher ratios, e.g., 8 for ciprofloxacin, are reached. Similarly a Cmax/MIC ratio for methicillin-susceptible S. aureus of 18 was obtained for serum and a ratio of 6 was obtained for muscle and subcutaneous tissue. Cmax/MIC ratios may thus differ significantly between the central and peripheral compartments, reaching effective ratios in one and ineffective ratios in another. Prediction of clinical outcome by pharmaco-kinetic surrogate markers has almost exclusively been performed by recalculation from concentrations in serum (Ryan et al., 1986; Muller et al., 1996).

Norfloxacin being a quinolone primarily concentrated intra-cellularly. However, the sequestered quinolone serves as a reservoir to prolonged exposure of bacteria in the extracellular compartments and to extend the half-life in serum. Therefore clinical dosages against intracellular pathogens should also be selected to achieve target concentrations in serum in relation to the bacterial MIC or MBC (Nix et al., 1991a). The quinolones have very large volumes of distribution and high tissue/serum ratios, apparent even after a single dose. Norfloxacin is more lipid soluble and its serum protein binding is less than 40 per cent., hence widely distributed throughout the body. The large volumes of distribution primarily reflect the large fraction of the total body load (at least 60%) which is inside cells (Nix et al., 1991b). Norfloxacin possess short half-life in rabbits, hence a dosage regimen of 80 and 77 mg.kg-1 at 12 h interval required in order to maintain therapeutic concentration of 0.5 µg.ml-1 plasma (Pavithra et al., 2009). Optimal antibacterial activity with fluoroquinolones is achieved by peak serum concentrations approximately ten times greater than the MIC and with the added advantage of lower MIC of norfloxacin for certain important gram negative bacterial pathogens, per os administration in rabbits is able to achieve optimal peak concentration to exert bactericidal effect. Considering PK-PD integration and the last detected antibiotic concentration (Clast 0.015µg.ml-1), it is suggested that norfloxacin could be a potential candidate primarily to treat serious infections caused by gram negative pathogens rather than gram positive infections in rabbits.

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