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Clinical pharmacokinetics of norfloxacin-glycine acetate after intravenous and oral administration in pigs

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The pharmacokinetics and dosage regimen of norfloxacin-glycine acetate (NFLXGA) was investigated in pigs after a single intravenous (i.v.) or oral (p.o.) administration at a dosage of 7.2 mg/kg body weight. After both i.v. and p.o. administration, plasma drug concentrations were best fitted to an open two-compartment model with a rapid distribution phase. After i.v. administration of NFLXGA, the distribution ($t_{1/2\alpha}$) and elimination half-life ($t_{1/2\beta}$) were 0.36 ± 0.07 h and 7.42 ± 3.55 h, respectively. The volume of distribution of NFLXGA at steady state (Vd_{ss}) was 4.66 ± 1.39 l/kg. After p.o. administration of NFLXGA, the maximal absorption concentration (C_{max}) was 0.43 ± 0.06 μg/ml at 1.36 ± 0.39 h (T_{max}). The mean absorption ($t_{1/2ka}$) and elimination half-life ($t_{1/2\beta}$) of NFLXGA were 0.78 ± 0.27 h and 7.13 ± 1.41 h, respectively. The mean systemic bioavailability (F) after p.o. administration was 31.10 ± 15.16%. We suggest that the optimal dosage calculated from the pharmacokinetic parameters is 5.01 mg/kg per day i.v. or 16.12 mg/kg per day p.o.

Key words: norfloxacin, pharmacokinetics, pig

Introduction

Fluoroquinolones are a group of synthetic antimicrobial agents that are highly potent and exhibit a broad spectrum of activity against a variety of mycoplasmas and Gram-negative bacteria, and some Gram-positive bacteria [5,11]. Norfloxacin is one of the first modern fluoroquinolone antimicrobial agents featuring a fluorine atom in position 6 and a piperazinyl or pyrrolidinyl substituent in position 7 of the quinoline nucleus [24]. Norfloxacin-glycine acetate (NFLXGA), a newly formulated norfloxacin that exerts its antibacterial effect by breaking double-stranded DNA [15], has been widely used for both prevention and therapeutic treatment of bacterial infections in humans and animals.

The quinolones bear both an acidic group (carboxylic acid) and a basic group (tertiary amine). This association gives them amphoteric properties. Their lipid solubility is low, except between pH 6 and 8. Within this range they have low water solubility and are prone to precipitate under more acidic conditions [22]. In order to overcome this problem, we made a new salt form, NFLXGA [15]. NFLXGA has a high solubility in water and it did not precipitate under acidic conditions ranging from pH 4 to 7 over a 6 month period (data not shown).

The pharmacokinetics of norfloxacin have been studied in various animals including dogs [4], pigs [2], chickens [3,12], calves [9] and laboratory animals [7]. The optimal dose range of the drug has been suggested to be 5-22 mg/kg body weight in these animals, on the basis of the minimal inhibition concentration (MIC) and the maximal norfloxacin concentration (C_{max}) in blood following drug administration. In our previous studies, we reported the pharmacokinetics of NFLXGA in flounder [16], horse [17] and rabbits [18].

In recent years, it has been suggested that the optimal dosage should be set in terms of pharmacokinetic-pharmacodynamic (PK/PD) relationships [20]. The pharmacokinetics of NFLXGA after oral administration has not been established in pigs. Therefore, the present study was designed to provide the clinical pharmacokinetics of norfloxacin following intravenous (i.v.) and oral (p.o.) administration in pigs and to determine the optimal dosage on the basis of the PK/PD parameters.

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Materials and Methods

Animals

Six male pigs weighing 60 ± 5 kg were used in this study. The animals were reared and maintained at the Chungnam National University Farm. They were housed indoors and fed with a drug-free commercial pellet diet and water *ad libitum*. The Animal Ethics Committee of the Veterinary Faculty at Chungnam National University approved the study.

Treatment

The study was carried out in a two-period crossover manner with animals randomly divided into two groups of three pigs. In period 1, three pigs received NFLXGA (Daesung Microbiologicals, Korea) i.v. over 40 sec at a dose of 7.2 mg/kg whilst three other pigs received the same dose of norfloxacin p.o. The formulation of NFLXGA consisted of norfloxacin (0.75 parts), acetic acid (0.15 parts) and glycine (0.1 part) based on mass. After an interval of 21 days, the treatments were reversed, i.e., pigs that previously received NFLXGA i.v. were administered the drug p.o., and those that initially received NFLXGA p.o. were administered the drug i.v. in period 2.

Blood sampling

5 ml blood samples were collected from the jugular vein directly into tubes before (0 h) and 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 h following drug administration. The serum samples were separated by centrifugation at 8,000 g for 5 min and were stored at −70°C (for up to one week) until determination of the norfloxacin concentration.

Norfloxacin analysis

The amount of norfloxacin was measured by high performance liquid chromatography (HPLC) using the method described previously by Park *et al*. [18]. Briefly, 1 ml serum was deproteinated with 1 ml 20% cold trichloroacetic acid in methanol. The mixture was vortexed for 1 min and centrifuged for 5 min at 15,000 g. 20 μl of each supernatant was injected into a HPLC system equipped with a reverse phase column (particle size 10 μm; 30 cm × 3.6 mm) and measured at a UV wavelength of 278 nm. The mobile phase was composed of 20% citric acid, 0.01 M phosphate buffer containing 1 mM heptane sulfonic acid and acetonitrile (800 : 1 : 200 = v/v/v) and the pH was adjusted to 3.0 with phosphoric acid. The validated limit of norfloxacin quantification for this method was 0.05 μg/ml. The extraction recoveries were greater than 80% and the coefficient of variation was less than 10% indicating high reproducibility.

Data analysis and dosage regimen

All pharmacokinetic parameters were derived using the WinNonlin software package (SCI, USA). The individual serum concentration data following administration were analyzed by nonlinear least-squares regression analysis. The best fit was achieved with a two-compartment model for both i.v. and p.o. administration. As a result, the serum concentration time curves of norfloxacin after a single i.v. or p.o. dose were fitted to the following equations:

$C_{i.v.} = Ae^{-\alpha t} + Be^{-\beta t}$

$C_{p.o.} = Ae^{-\alpha t} + Be^{-\beta t} - Ce^{-kat}$

where ($C_{i.v.}$) and ($C_{p.o.}$) are the concentrations in serum at time t after i.v. and p.o. administration respectively; A and B are the zero-time serum drug concentration intercepts of biphasic i.v. and p.o. disposition curves; C is the zero-time serum drug concentration intercept of the absorption phase after p.o. administration; e is the base of the natural logarithm; α is the hybrid rate constant of the slope of distribution phase; β is the hybrid rate constant of the slope of elimination phase; and k_a is the hybrid rate constant of the slope of absorption. Following p.o. administration, the bioavailability F was calculated according to the equation:

$F\ (\%) = (AUC_{p.o.}/AUC_{i.v.}) \times (\beta_{p.o}/\beta_{i.v.}) \times 100\%$.

The equations used for calculating dosage in pigs were as follows:

$Dose_{iv} = C_{ave} \times Clearance = C_{ave} \times Vd_{ss} \times (0.693/t_{1/2\beta})$;

$Dose_{po} = Dose_{iv}/F$.

Results

The concentrations-time curves of norfloxacin following single i.v. and p.o. administration of 7.2 mg NFLXGA/kg body weight to pigs are shown in Fig. 1. Concentration versus time data were analyzed to achieve the best fit with a two-compartment model after both routes of administration in all pigs. The pharmacokinetic parameters are summarized in Table 1.

15 min after i.v. and p.o. administration of NFLXGA, the serum concentrations of norfloxacin were 5.22 ± 1.40 μg/ml and 0.18 ± 0.08 μg/ml, respectively (Fig. 1.). Thereafter, serum norfloxacin concentrations were maintained in all animals for up to 24 h at more than 0.05 ± 0.03 μg/ml (i.v.) and 0.03 ± 0.02 μg/ml (p.o.). The distribution rate constant was 1.96 ± 0.36 h after i.v. administration with a distribution half-life ($t_{1/2\alpha}$) of 0.36 ± 0.07 h.

The serum concentration of norfloxacin reached a maximum level (C_{max}) of 0.43 ± 0.06 μg/ml at 1.36 ± 0.39 h (T_{max}), and the absorption half-life ($t_{1/2ka}$) was 0.78 ± 0.27 h after p.o. administration in pigs. The mean elimination half-lives ($t_{1/2\beta}$) after i.v. and p.o. administration were 7.42 ± 3.55 h and 7.13 ± 1.41 h respectively, and there are no significant differences. The systemic bioavailability (F) after oral administration of NFLXGA was 31.10 ± 15.16%.

Table 1. Parmacokinetic parameters that describe the disposition of norfloxacin-glycine acetate (7.2 mg/kg body weight) after intravenous and oral administration in six pigs

Parameters	Unit	I.V. administration (Mean ± SD)	P.O. administration (Mean ± SD)
A	μg/ml	7.85 ± 2.75	41.2 ± 14.09
B	μg/ml	0.58 ± 0.16	0.24 ± 0.10
α	/h	1.96 ± 0.36	0.99 ± 0.38
β	/h	0.12 ± 0.06	0.09 ± 0.03
AUC	μg/ml · h	9.66 ± 2.55	3.47 ± 1.11
$t_{1/2ka}$	h	—	0.78 ± 0.27
$t_{1/2\alpha}$	h	0.36 ± 0.07	0.79 ± 0.29
$t_{1/2\beta}$	h	7.42 ± 3.55	7.13 ± 1.41
k_{12}	/h	0.93 ± 0.17	0.47 ± 0.21
k_{21}	/h	0.26 ± 0.16	0.30 ± 0.18
C_{max}	μg/ml	—	0.43 ± 0.06
T_{max}	h	—	1.36 ± 0.39
CLB	l/kg/h	0.8 ± 0.26	—
AUMC	μg · h^2/ml	68.17 ± 49.08	—
Vd_{SS}	l/kg	4.66 ± 1.39	—
F	%	—	31.10 ± 15.16

The results were expressed as mean ± SD (n = 6). *A* and *B*, zero-time serum concentration intercepts of biphasic i.v. and p.o. disposition curves; α, hybrid rate constants of the slope of distribution; β, hybrid rate constants of the slope of elimination; AUC, the area under the concentration-time curves; $t_{1/2ka}$, the absorption half-life; $t_{1/2\alpha}$, the distribution half-life; $t_{1/2\beta}$, the elimination half-life; k_{12} and k_{21}, first-order transfer rate constants for drug distribution from the central compartment to the peripheral compartment and from the peripheral compartment to the central compartment; C_{max}, maximum concentration; T_{max}, time to reach the maximum concentration; MRT, mean residence time; CLB, serum clearance; AUMC, total area under the moment curve; Vd_{SS}, steady-state volume of distribution; F, bioavailability.

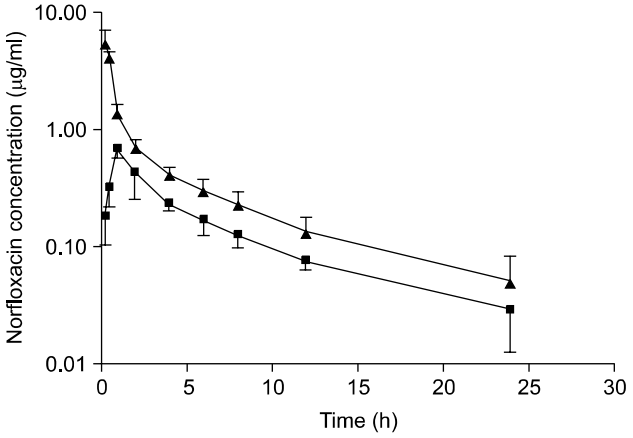

Fig. 1. Serum concentration of norfloxacin following i.v. (▲) or p.o. (■) administration of 7.2 mg NFLXGA per kg body weight (n = 6).

Discussion

The mean elimination half-life ($t_{1/2\beta}$) after i.v. administration of NFLXGA in the present study was estimated to be 7.42 ± 3.55 h. This is longer than the $t_{1/2\beta}$ in rabbits (3.93 ± 1.54 h) [18] or horses (5.44 ± 1.36 h) [17], and also longer than that found in the previous studies of norfloxacin in pigs (3.65 ± 0.16 h) [2] and dogs (3.56 h) [4], and that of norfloxacin nicotinate in swine (2.1 h) [21] and donkeys (3.51 ± 0.49 h) [13]. It is however a little shorter than that seen in chickens (8.0 ± 0.3 h) [3]. The volume of distribution at steady state (Vd_{SS}, 4.66 ± 1.39 l/kg) was higher than the previously reported value (2.21 ± 0.21 l/kg) [2], and the ratio of k_{12} and k_{21} was 3.58. All of these findings suggested that the drug was well distributed and retained in the tissues. After p.o. administration of NFLXGA, the mean elimination half-life ($t_{1/2\beta}$) was 7.13 ± 1.41 h, similar to that obtained after i.v. administration. It has been reported that the systemic bioavailability (F) of norfloxacin is only 30 to 40% after p.o. administration [14]. In the present study, F was calculated to be about 31.10%, which is lower than the values determined in rabbits (40%) [18], and in broiler chickens (57.0%) [3], but is significantly higher than the oral bioavailability of norfloxacin nicotinate in donkeys with F values of 9.6% and 6.4% for the 10 and 20 mg/kg doses respectively [13]. In pigs, however we could not find the optimal dosage using PK and PD parameters.

The optimal dosage of drug can be determined with the equation provided by Toutain *et al*. [22], which is related to PK and PD parameters. In addition, Schentag stated previously that the AUC/MIC (AUIC) ratio of quinolones should be more than 125 to prevent selective pressure that leads to the overgrowth of resistant bacterial sub-populations [20]. The MIC of norfloxacin has been shown to be below 0.12 μg/ml for *Escherichia coli, Salmonella spp., Klebsiella pneumonia, Klebsiella oxytoca* and *Proteus vulgaris* [13]. Therefore, a desired average serum norfloxacin concentration of 0.48 μg/ml was selected by quadrupling the average MIC values in the present study. We suggest that the appropriate dosage of 5.01 mg/kg for i.v. and 16.12 mg/kg for p.o. per day or 2.51 mg/kg for i.v. and 8.06 mg/kg for p.o. per 12 h would provide a serum concentration in pigs high enough to inhibit bacteria with a MIC less than 0.12 μg/ml. However, NFLXGA should not be considered the drug of choice for pigs infected with pathogenic bacteria, such as *Streptococcus spp*. (MIC, 6.25 μg/ml), *Staphylococcus spp.* (MIC, 1.56 μg/ml), *Rhodococcus spp*. (MIC, 6.26 μg/ml), and *Bordetella spp*. (MIC, 3.12 μg/ml) showing more than 0.25 μg/ml of MIC [13,19].

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