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Bioavailability of norfloxacin from PEG 6000 solid dispersion and cyclodextrin inclusion complexes in rabbits

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Abstract

A comparative bioavailability study was carried out in rabbits on pure powder of norfloxacin and its formulations: aqueous solution, polyethyleneglycol 6000 solid dispersions (PEG 6000 SD), beta-cyclodextrin (β-CD) and hydroxypropyl-beta cyclodextrin (HP-β-CD) complexes. Norfloxacin plasma concentrations were measured by HPLC method with a fluorimetric detection. Estimation of $t_{1/2}$ and k_e proved that PEG 6000 SD and CD complexes did not modify the elimination characteristics of norfloxacin. Data from plasma concentration profiles indicated that absorption of norfloxacin from of SD and inclusion complexes was markedly accelerated when compared with powder of pure drug. The extent of absorption was significantly smaller with powder of norfloxacin than with its formulations. Bioavailability was improved and significantly higher with CD and complexes SD than with powder, but the improvement was lower than expected.

Keywords: Norfloxacin; β-cyclodextrin; Hydroxypropyl-β-cyclodextrin; PEG 6000; Solid Dispersion; Bioavailability

In the last decades, many techniques including preparation of solid dispersions (SD) (Francés et al., 1991; Sheen et al., 1991; Bhattacharyya et al., 1993; Kedzierewicz et al., 1993; Chowdary and Suresh Babu, 1994; Guyot et al., 1995) and cyclodextrin (CD) inclusion complexes (Otero-Espinar et al., 1991; Kedzierewicz et al., 1993; Yazan and Sumnu, 1994) have been tried, in order to improve the solubility and/or bioavailability of poorly water soluble drugs. The purpose of this work was to evaluate the influence of these techniques on the bioavailability of norfloxacin, after oral administration to rabbits of polyethyleneglycol 6000 (PEG 6000) SD, beta-cyclodextrin (β-CD) and hydroxypropyl-beta cyclodextrin (HP-β-CD) complexes.

Solid dispersion was prepared according to the procedure reported by Francés et al. (1991). Briefly, 2 g of PEG 6000 (Merck, Germany) were melted and added to 0.5 g of norfloxacin (Sigma, Saint-Louis, USA) while stirring. When an homogeneous mixture was attained, it was cooled to

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room temperature, ground, sieved and particles larger than 180 μm were discarded. The final SD contained 20% (w/w) of norfloxacin. CD inclusion complexes were prepared as previously described (Guyot et al., 1995) by dispersing at room temperature an excess of norfloxacin in an aqueous solution of CD using magnetic stirring (24 h). The suspension was then filtered and lyophilized. Particles larger than 180 μm were discarded by sieving. Thus, two CD formulations were prepared: norfloxacin/β-CD and HP-β-CD complexes containing 19.91% and 20.19% (w/w) of norfloxacin, respectively. Solution of norfloxacin (10 mg/ml) was obtained by dissolving pure drug in an aqueous solution of acetic acid (pH 4.5). Drug content of the formulations was assayed spectrophotometrically at 274 nm.

In vivo investigation was carried out on male albinos rabbits, weighing 2–2.5 kg, randomly assigned in five groups ($n = 6$) and fasted 18 h prior and 24 h following oral administration (however, water was allowed ad libitum). Five norfloxacin-formulations (powder and aqueous solution of pure drug, PEG 6000 SD, β-CD and HP-β-CD complexes) were given to rabbits (one formulation each group) by intubation with a plastic gastric tube. SD, CD complexes and powder of pure norfloxacin were previously filled into hard gelatin capsules. After intake of the drug, 10 ml of water were injected into the plastic tube. The aqueous solution of norfloxacin was previously diluted with water in order to administrate the total dose in a fixed volume of 10 ml.

Whatever norfloxacin formulation, a single dose of 10 mg/kg was given orally to each animal. After administration of norfloxacin formulations, blood samples (0.5 ml) were taken at defined times up to 24 h from all animals by marginal ear vein ponction and collected in heparinized tubes.

Plasma was isolated by centrifugation, frozen and stored at -25°C until further processing. Drug concentrations in plasma were determined employing a modified HPLC method (Myers and Blumer, 1987) using fluorimetric detection. Separation was achieved at room temperature on a prepacked Nucleosil C_{18} column. The mobile phase was a (78:13:9 v/v) mixture of 0.02 M dihydrogenpotassium phosphate, methanol and acetonitrile, containing 0.15% (w/v) of tetrabutylammonium hydroxide. The pH was adjusted to 3 $\pm$ 0.03 with o-phosphoric acid. The flow rate was 1.6 ml/min. Detection was performed with excitation wavelength set at 380 nm and emission wavelenght at 450 nm.

C_{max} and T_{max} were extracted from data. Area under the plasma level curves were calculated using the linear trapezoïdal rule and extrapolated to infinity. Elimination rate constant (k_e) and biological half-life ($t_{1/2}$) were calculated from the slope of the linear regression line in the elimination phase of the semi-logarithmic plot of plasma concentration v/s time. Percentage of drug absorbed at 0.75 h and 1.25 h were calculated by applying Wagner-Nelson's method to time versus plasma concentrations. Results were expressed by mean $\pm$ standard deviation. Statistical analysis was performed using Student t-test. Mean differences were considered significant at level $P < 0.05$.

As shown in Fig. 1, immediately after C_{max} was attained, plasma concentrations decreased quickly. This phenomenon was more progressive with powder of pure norfloxacin and in agreement with findings reported by Kedzierewicz et al. (1993) for tolbutamide after administration to rabbits and attributed to the rapid release of drug. After C_{max}, the absorption process was already over and plasma concentrations display only the apparent elimination of norfloxacin.

Using norfloxacin solution as reference, results indicated that absorption from solid preparations was slower. The lowest value (40%) resulted from SD (Table 1). Differences in mean $AUC^{0-0.75}$ were also statistically significant between norfloxacin solution and its solid formulations except β-CD complex (Table 3). Seventy five minutes after administration of norfloxacin formulations, absorption was faster with SD and CD complexes than with powder (Table 1), but differences in the mean $AUC^{0-1.25}$ remained statistically not different between the four norfloxacin solid preparations (Table 2).

Mean AUC^{0-24} and $AUC^{0-\infty}$ with pure drug were significantly different ($P < 0.05$) of those obtained with the four other formulations (Table 2). However, statistical analysis did not reflecte a

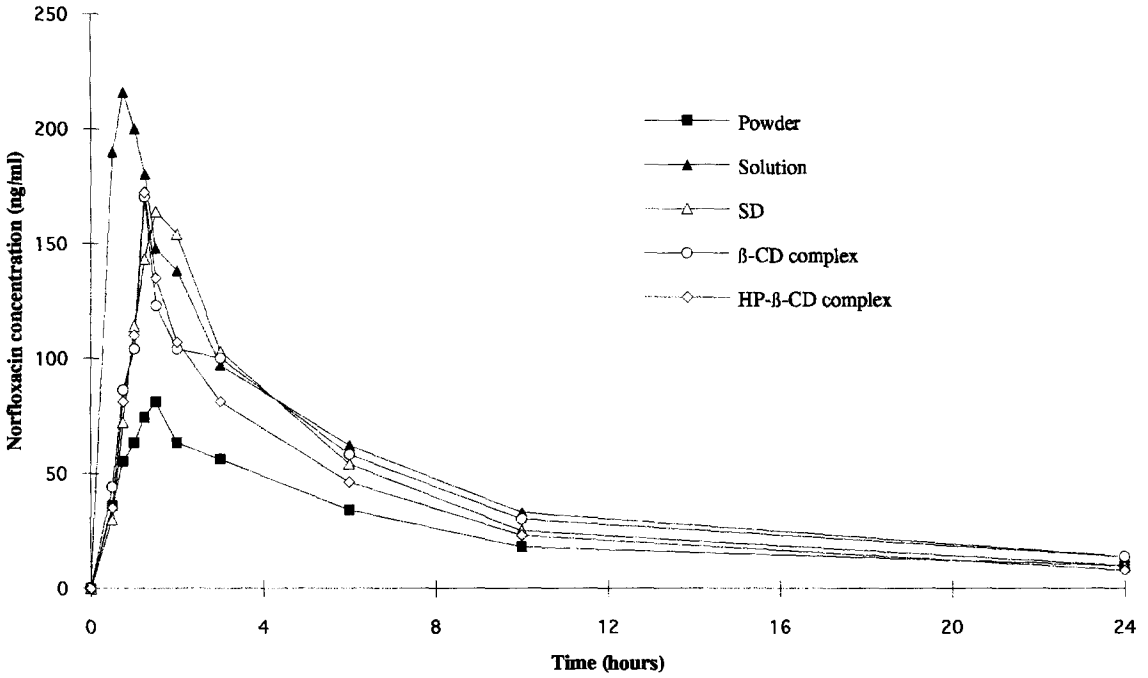

Fig. 1. Mean plasma concentration-time profiles of norfloxacin after oral administration of powder, solution, PEG 6000 solid dispersion and CD complexes of norfloxacin at a dose of 10 mg of drug/kg to rabbits.

major difference neither between the mean AUC^{0-24} nor between the mean $AUC^{0-\infty}$ following the administration of solution, SD and β-CD complex (Table 3). Such results indicated, on one hand, that the extent of bioavailability was significantly lower from the powder of pure drug than from the other preparations and, on the other hand, that both, PEG 6000 SD and CD complexes, improved norfloxacin oral bioavailability.

Differences were also evident in the kinetic part of bioavailability. The highest norfloxacin plasma concentration was attained at t_{max} = 0.75 h with the solution (Table 1). The lowest mean C_{max} was obtained after administration of powder of pure drug and t_{max} was then delayed from 0.70 h as compared with solution. Differences in mean C_{max} and t_{max} were statistically significant between pure norfloxacin and both CD complexes but not between pure drug and its PEG 6000 SD (Table 2). This is likely due to the larger variability of absorption with the SD as reflected in the higher intersubject coefficients of variation (CV%) for SD data (Table 1). This result is not in agreement with that reported from oral administration of tolbutamide to rabbit where mean C_{max} was higher with powder of pure drug than with solution, SD and CD complexes due to the decrease in aqueous solubility of tolbutamide in gastric medium allowing its precipitation (Kedzierewicz et al., 1993), whereas gastric medium is suitable to maintain norfloxacin in solution. Furthermore, if differences in mean C_{max} were not significant between solution, SD and CD complexes, on the contrary, as shown in Table 3, differences in mean t_{max} were very significant between solution and the four norfloxacin solid preparations. This result indicated that absorption had nearly the same extent with solution, SD and CD complexes but was significantly slower with all norfloxacin solid preparations as compared with solution.

These results could be related to those obtained in our previous in vitro investigation (Guyot et al., 1995), where it was found that CD complexes and PEG 6000 SD improved dissolution properties of norfloxacin. As norfloxacin is poorly soluble in water, its accelerated absorption rate with

Table 1
Pharmacokinetic and bioavailability parameters obtained after oral administration to rabbits of a dose equivalent to 10 mg/kg of the five norfloxacin preparations (powder and solution of pure drug, SD and inclusion complexes)

Parameters	Powder of pure norfloxacin	Solution of pure norfloxacin	Solid dispersion (SD)	β-CD Complex	HP-ß-CD Complex
C_{max} (ng/ml)	82 ± 26 (32)	245 ± 121 (49)	178 ± 94 (53)	170 ± 77 (45)	172 ± 83 (48)
t_{max} (h)	1.45 ± 0.11 (8)	0.75 ± 0.27 (36)	1.45 ± 0.11 (8)	1.25 (0)	1.29 ± 0.10 (8)
$AUC^{0-0.75}$ (ng·h/ml)	20 ± 14	98 ± 70	20 ± 13	27 ± 17	23 ± 11
$AUC^{0-1.25}$ (ng·h/ml)	52 ± 30	198 ± 110	76 ± 30	85 ± 50	82 ± 30
AUC^{0-24} (ng·h/ml)	599 ± 56 (31)	1187 ± 364 (9)	964 ± 253 (26)	996 ± 230 (23)	812 ± 176 (22)
$AUC^{0-\infty}$ (ng·h/ml)	668 ± 78 (11)	1277 ± 356 (30)	1030 ± 265 (26)	1084 ± 206 (19)	857 ± 168 (19)
Relative bioavailability (%)	49 ± 6 (12)	100	67 ± 18 (27)	71 ± 17 (24)	59 ± 9 (15)
$t_{1/2}$ (h)	4.54 ± 1.11 (24)	4.40 ± 0.84 (19)	4.46 ± 1.55 (35)	4.35 ± 1.25 (29)	4.13 ± 0.93 (23)
K_e (h^{-1})	0.1608 ± 0.0410 (25)	0.1616 ± 0.0261 (16)	0.1862 ± 0.0603 (32)	0.1693 ± 0.0442 (26)	0.1827 ± 0.0438 (24)
Percentage of drug absorbed in 0.75 h	55	100	40	49	55
Percentage of drug absorbed in 1.25 h	77	100	83	100	100

C_{max}, maximum plasma concentration; t_{max}, time to reach C_{max}; $AUC^{0-0.75}$, area under the plasma level curve between time 0 and 0.75 h; $AUC^{0-1.25}$, area under the plasma level curve between time 0 and 1.25 h; AUC^{0-24}, area under the plasma level curve between time 0 and 24 hours; $AUC^{0-\infty}$, total area under the plasma level curve; $t_{1/2}$, terminal half-life; k_e, constant of elimination rate; between brackets are indicated the intersubject coefficients of variation (CV%).

Table 2
Statistical comparison (*P* values) of norfloxacin bioavailability parameters between powder of pure norfloxacin and its preparations following oral administration of a unique dose of 10 mg/kg to rabbits

Parameters	Solution of norfloxacin	Solid dispersion	β-CD complex	HP-β-CD complex
C_{max}	<0.05	NS	<0.05	<0.05
t_{max}	<0.001	NS	<0.01	<0.05
$AUC^{0-0.75}$	<0.05	NS	NS	NS
$AUC^{0-1.25}$	<0.05	NS	NS	NS
AUC^{0-24}	<0.05	<0.05	<0.05	<0.05
$AUC^{0-\infty}$	<0.01	<0.05	<0.01	<0.05

C_{max}, maximum plasma concentration; t_{max}, time to reach C_{max}; $AUC^{0-0.75}$, area under the plasma level curve between time 0 and 0.75 h; $AUC^{0-1.25}$, area under the plasma level curve between time 0 and 1.25 h; AUC^{0-24}, area under the plasma level curve between time 0 and 24 h; $AUC^{0-\infty}$, total area under the plasma level curve; NS = not significant.

CD complexes and SD was probably due to the increase in its dissolution rate. However, it is surprising that mean $AUC^{0-\infty}$ and relative bioavailability found with HP-β-CD complex, which had the most rapid in vitro dissolution rate, were lower than those found with β-CD complex and SD. On the other hand, the improvement of norfloxacin bioavailability was lower than expected (Table 1). This result confirmed findings already reported for indomethacin/β-CD complex (Szejtli, 1988) and naproxen/β-CD complex (Otero-Espinar et al., 1991). The explanation for

Table 3
Statistical comparison (P values) of norfloxacin bioavailability parameters between norfloxacin solution and the four norfloxacin solid preparations (powder of pure drug, SD and inclusion complexes) following oral administration of a unique dose of 10 mg/kg to rabbits

Parameters	Powder of norfloxacin	Solid dispersion	β-CD complex	HP-β-CD complex
C_{max}	<0.05	NS	NS	NS
t_{max}	<0.001	<0.001	<0.05	<0.01
$AUC^{0-0.75}$	<0.05	<0.05	NS	<0.05
$AUC^{0-1.25}$	<0.05	<0.05	NS	<0.05
AUC^{0-24}	<0.05	NS	NS	<0.05
$AUC^{0-\infty}$	<0.01	NS	NS	<0.05
Relative bioavailability	<0.05	NS	NS	NS

C_{max}, maximum plasma concentration; t_{max}, time to reach C_{max}; $AUC^{0-0.75}$, area under the plasma level curve between time 0 and 0.75 hour; $AUC^{0-1.25}$, area under the plasma level curve between time 0 and 1.25 h; AUC^{0-24}, area under the plasma level curve between time 0 and 24 h; $AUC^{0-\infty}$, total area under the plasma level curve; NS = not significant.

this is that aqueous solubility is probably not the unique limiting step in the absorption of norfloxacin.

$t_{1/2}$ was found to be nearly the same after administration of all norfloxacin formulations indicating that PEG 6000 SD and CD complexes did not alter the elimination characteristics of norfloxacin. This result is in agreement with that reported by Chowdary and Suresh Babu (1994) for indomethacin SD after oral administration to human subjects. However, Kedzierewicz et al. (1993) have found that half-life of tolbutamide was nearly the same after intravenous administration of solution and oral administration of CD complex to rabbit, but was longer after administration of SD.

In conclusion, the results of the present study demonstrated that both PEG 6000 SD and CD complexes provided superior norfloxacin oral bioavailability as compared to powder of pure drug. However, in considering the increase in the in vitro solubility and dissolution rate of norfloxacin, bioavailability improvement with SD and CD complexes was less dramatic than expected because the poor aqueous solubility was not the unique explanation of the low oral bioavailability of norfloxacin.

References

Bhattacharyya, M., Basu, S.K., Gupta, B.K., Ghosal, S.K., Mandal, S.C. and Chattaraj, S.C., Formulation and in vitro-in vivo charaterization of solid dispersions of piroxicam. *Drug Dev. Ind. Pharm.*, 19 (1993) 739–747.

Chowdary, K.P.R. and Suresh Babu, K.V.V., Dissolution, bioavailability and ulcerogenic studies on solid dispersions of indomethacin in water soluble cellulose polymer. *Drug Dev. Ind. Pharm.*, 20 (1994) 799–813.

Francés, C., Veiga, M.D., Español, O.M. and Cadorniga, R., Preparation, characterization and dissolution of ciprofloxacin/PEG 6000 binary systems. *Int. J. Pharm.*, 77 (1991) 193–198.

Guyot, M., Fawaz, F., Bildet, J., Bonini, F. and Lagueny, A.M., Physicochemical characterization and dissolution of norfloxacin/cyclodextrin inclusion compounds and PEG solid dispersions. *Int. J. Pharm.*, 123 (1995) 53–63.

Kedzierewicz, F., Zinutti, C., Hoffman, M. and Maincent, P., Bioavailability study of tolbutamide β-cyclodextrins inclusion compounds, solid dispersions and bulk powder. *Int. J. Pharm.*, 94 (1993) 69–74.

Myers, C.M. and Blumer, J.L., High-performance liquid chromatography of ciprofloxacin and its metabolites in serum, urine and sputum. *J. Chromatogr.*, 422 (1987) 153–164.

Otero-Espinar, F.J., Anguiano-Igea, S., Garcia-Gonzalez, N., Vila-Jato, J.L. and Blanco-Mendez, J., Oral bioavailability of naproxen-β-cyclodextrin inclusion compound. *Int. J. Pharm.*, 75 (1991) 37–44.

Sheen, P.C., Kim, S.I., Petillo, J.J. and Serajuddin, A.T.M., Bioavailability of a poorly water-soluble drug from tablet and solid dispersion in humans. *J. Pharm. Sci.*, 80 (1991) 712–714.

Szejtli, J., *Cyclodextrin Technology*, Kluwer, Dordrecht, The Netherlands, 1988.

Yazan, Y. and Sumnu, M., Improvement in the dissolution properties of theophylline with β-cyclodextrin. *S.T.P. Pharma Sci.*, 4 (1994) 128–132.