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Short Communication

Ciprofloxacin-loaded polyisobutylcyanoacrylate nanoparticles: pharmacokinetics and in vitro antimicrobial activity

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Abstract

The disposition of ciprofloxacin in free form and loaded PIBCA/NP has been studied after intravenous infusion to the rabbit. Data from plasma concentration profiles revealed that pharmacokinetic parameters of ciprofloxacin associated with the colloidal carrier were greatly modified. Thus, ciprofloxacin-loaded PIBCA/NP led to increased AUC, $t_{1/2}$ and V_d, and to a decreased Cl as compared with drug in solution. This could be due not only to the colloidal drug carrier but also to the pharmacokinetics of ciprofloxacin itself. Studies of efficacy against *Mycobacterium avium* complex in human macrophages proved that ciprofloxacin-loaded PIBCA/NP was more effective than free drug. The cytotoxicity of the polymeric material was observed at concentrations higher than 80 μg of PIBCA per ml with drastic reduction of viable macrophages. This may explain why the efficacy of ciprofloxacin associated with nanoparticles was much lower than expected.

Keywords: Antimicrobial activity; Ciprofloxacin; Intracellular infections; Pharmacokinetics; Polyisobutylcyanoacrylate

Ciprofloxacin, a monofluorinated quinolone, has a wide spectrum of activity. Its efficiency has led to its use being proposed for the treatment of various microbial diseases. However, the ability to treat intracellular infections may depend on the concentration of the drugs inside macrophages (Majumdar et al., 1992). It has been proposed that association of antibiotics with colloidal drug carriers allows intracellular targeting because they are taken up by the reticuloendothelial system (RES). Therefore, many antibiotics were associated with liposomes (Majumdar et al., 1992; Magallanes et al., 1993) and polymeric nanoparticles (Henry-Micheland et al., 1987; Alonso et al.,

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1991; Fresta et al., 1995). In this work, the disposition of ciprofloxacin-loaded polyisobutylcyanoacrylate nanoparticles (PIBCA/NP) was studied after intravenous (iv) infusion to rabbits and its efficacy against *Mycobacterium avium* complex (MAC) in human macrophages was evaluated.

Ciprofloxacin-loaded PIBCA/NP and blank NP (ciprofloxacin free) were prepared, drug content (1 mg/10 mg PIBCA/NP) determined and mean overall particle size (195 ± 52 nm) measured as previously described (Fawaz et al., 1997).

Investigation in vivo was carried out on male albinos rabbits, weighing 2–2.5 kg, randomly assigned in two groups ($n = 8$). Two ciprofloxacin preparations (solution and PIBCA/NP) were given to animals (one preparation each group) by i.v. infusion at a constant rate over 1 h via the marginal ear vein. Drug was given to rabbits in single doses of 10 mg/kg in a fixed volume (10 ml). At defined times, blood samples (0.5 ml) were taken (up to 24 h) from all animals from the marginal ear vein and collected in heparinized tubes. Plasma was isolated by centrifugation (3000 rpm) frozen and stored at -25°C until further processing. Drug concentrations in the plasma were determined employing a modified HPLC method previously described (Fawaz et al., 1996) using fluorimetric detection (380 nm excitation, 450 nm emission wavelengths) and enoxacin as internal standard. The half-life ($t_{1/2}$) was calculated from the terminal phase of the elimination curves. Area under the curves (AUC) was determined using the trapezoidal rule with extrapolation to infinity for the total AUC ($AUC_{0-\infty}$). The total body clearance (Cl) and the apparent volume of distribution (V_d) were calculated as:

$$Cl = \text{i.v. dose}/AUC_{0-\infty}$$

$$V_d = Cl/k_e$$

The efficacy of ciprofloxacin-loaded PIBCA/NP against five strains of MAC isolated from AIDS patients was evaluated in human blood monocyte-derived macrophages in comparison with solution of free drug and blank PIBCA/NP. Four ciprofloxacin concentrations (4, 8, 16 and 32 μg/ml) in the incubation medium were used. Macrophages were obtained from peripheral blood of healthy donors as previously described (Pellegrin et al., 1996). On day 7, macrophages were inoculated with 1 ml per well of a MAC suspension containing 1×10^6 bacteria/ml and incubated for 3 h at 37°C in 5% CO_2 atmosphere. At days 0 (after 3 h incubation), 4 and 7, the medium from alternate plates was discarded and the monolayers were lysed by introducing 1 ml of distilled water over 30 min and mechanical shaking. Samples of supernatant and lysates of macrophages were taken for cfu counts at days 0 (180 min after incubation), 4 and 7 after infection.

Statistical analysis was performed using t-test between mean for unpaired pharmacokinetic data. Significant differences were judged as $p < 0.05$.

Results from cfu counts are expressed as mean of two experiments (each one in duplicate) $\pm$ S.D. of $\log_{10}$ cfu/ml of lysate. They were compared on days 4 and 7 between controls, and each drug experiment by a one-way analysis of variance (ANOVA). A t-test was used to compare the means when F was significant. Mean differences were considered at level $p < 0.05$.

As shown in Fig. 1, plasma concentration–time profiles were very similar from both ciprofloxacin preparations. Plasma concentrations declined quickly at the end of i.v. infusion. At 24 h, small amounts of drug were found in the plasma of rabbits to which ciprofloxacin-loaded PIBCA/NP were given, whereas no drug was found in the plasma of rabbits to which ciprofloxacin solution was infused.

At the end of the i.v. infusion, mean plasma concentration (C_1) was slightly lower from solution than from nanoparticles. This result is not in agreement with previous reports after i.v. administration of poylalkylcyanoacrylates NP to mice (Grislain et al., 1983), i.v. infusion of indomethacin-loaded PIBCA nanocapsules to rats (Andrieu et al., 1989) and indomethacin-loaded polylactides nanocapsules to rabbits (Fawaz et al., 1996), and could be explained by the reduction of the colloidal carrier clearance from blood by the RES due to the Küpffer cells saturation. It should be noted that the mean ciprofloxacin plasma concentration at 0.25 h was significantly higher from nanoparticles than from solution. At 0.75 h, both

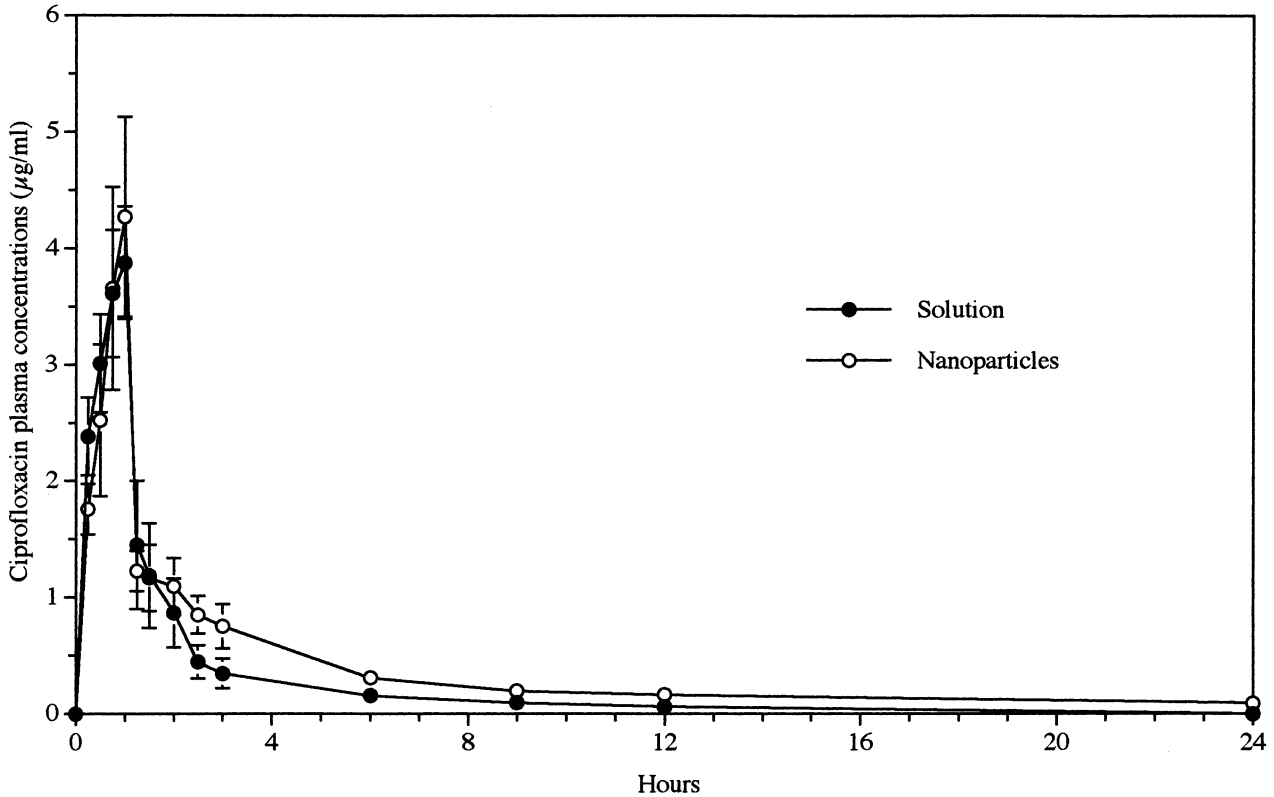

Fig. 1. Mean plasma concentration–time profiles following intravenous infusion of ciprofloxacin in solution and loaded poly-isobutylcyanoacrylate nanoparticles at a dose of 10 mg/kg to rabbits.

concentrations became practically equal. Then, one could suppose that at 0.25 h Küpffer cells saturation was not yet attained. The suppression of the RES by means of different agents has been used in order to achieve redistribution of the particles to other sites (Illum et al., 1986; Proffit et al., 1986). In our work, since the drug content of NP was 1 mg per 10 mg of PIBCA (3×10^{12} nanoparticles), the amount of nanoparticles administered to each rabbit was about 3×10^{13} per kg. It is then likely that the RES of the rabbit was temporarily saturated by the infused nanoparticles. On the other hand, a temporary decrease in the plasma concentrations has been reported at 1 min after i.v. infusion over 1 min of 30 mg/kg ciprofloxacin hydrochloride to rabbit and attributed to the low ciprofloxacin solubility in blood (Yoon et al., 1994). However, in our case, it is unlikely that solubility of ciprofloxacin would be a plausible explanation since the dose was lower (10 mg/kg) and was infused i.v. over a longer period of time (1 h).

Disposition parameters of ciprofloxacin were significantly modified after i.v. infusion of NP (Table 1). The increased $AUC_{0-\infty}$ may be explained by the prolonged release of ciprofloxacin from nanoparticles captured by the RES and by those remaining in the blood after RES was saturated. This result is not in agreement with those previously reported from indomethacin associated with colloidal carriers (Andrieu et al., 1989; Fawaz et al., 1996) where $AUC_{0-\infty}$ was significantly greater from i.v. infusion of the solution than from the colloidal carriers. It should be noted that the total plasma clearance was very significantly reduced with nanoparticles. Such a result suggests that association of ciprofloxacin with PIBCA/NP did affect the rate of elimination

Table 1
Pharmacokinetic parameters (mean ± S.D., $n = 8$) following intravenous infusion to rabbits of a dose equivalent to 10 mg/kg of ciprofloxacin in solution and loaded PIBCA nanoparticles

Pharmacokinetic parameters	Ciprofloxacin in solution	Ciprofloxacin-loaded PIBCA/NP	Statistical comparison (p values)
$C_{0.25}$ (μg/ml)	2.38 ± 0.33 (14)	1.77 ± 0.22 (12)	<0.001
$C_{0.50}$ (μg/ml)	2.98 ± 0.38 (13)	2.52 ± 0.65 (26)	NS
C_1 (μg/ml)	3.87 ± 0.48 (12)	4.27 ± 0.86 (20)	NS
AUC_{0-12} (μg·h/ml)	6.13 ± 1.34 (22)	7.85 ± 1.46 (19)	<0.05
AUC_{0-24} (μg·h/ml)	6.49 ± 1.44 (22)	9.38 ± 1.57 (17)	<0.005
$AUC_{0-\infty}$ (μg·h/ml)	6.55 ± 1.43 (22)	11.38 ± 1.41 (12)	<0.001
$t_{1/2}$ (h)	4.812 ± 0.690 (14)	15.162 ± 4.030 (27)	<0.001
Cl (l/h/kg)	1.58 ± 0.30 (19)	0.89 ± 0.11 (12)	<0.001
V_d (l/kg)	11.058 ± 2.90 (26)	19.774 ± 6.660 (34)	<0.005

$C_{0.25}$, $C_{0.50}$ and C_1, plasma concentrations at 0.25, 0.50 and 1 h respectively after the beginning of the intravenous infusion; AUC_{0-12}, area under the plasma level curve between time 0 and 12 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; $t_{1/2}$, terminal half-life; Cl, total clearance; V_d, apparent volume of distribution; NS, not significatif. The intersubject coefficients of variation (CV%) are indicated in parentheses.

of the drug. However, it must be emphasized that ciprofloxacin is 30% bound to serum proteins and has a relative short elimination half-life (Table 1). $t_{1/2}$ was 3-fold increased after i.v. infusion of ciprofloxacin-loaded PIBCA/NP as a result of the slow release of the drug from nanoparticles. The slow release of drug from the nanoparticles in the blood would also explain the increase in the V_d value. Ciprofloxacin has a good tissue diffusion, as can be deduced from the V_d value (11.06 ± 2.9 l/kg) and its tissue concentrations could be several times above the simultaneous plasma levels (Schlenkhoff et al., 1986; Rohwedder et al., 1991). Similar findings have been reported from rabbits after i.v. administration of ciprofloxacin (Yoon et al., 1994).

Table 2 summarizes the effects of different ciprofloxacin concentrations, on the MAC growth. Whatever the ciprofloxacin preparation, no effect was found at 4 μg/ml drug concentration. A concentration of 8 μg/ml of ciprofloxacin-loaded nanoparticles reduced the cfu by 1.1 and 1.2 log units at days 4 and 7, respectively, compared with day 0, while the free drug reduced the cfu by 0.65 and 0.85, respectively. As the concentration of ciprofloxacin increased in the range 4–32 μg/ml the cfu reduction from both ciprofloxacin preparations decreased with a better efficiency from NP ($p < 0.05$). However, this activity remained much lower than that reported from liposome-encapsulated ciprofloxacin against the MAC in human macrophages (Majumdar et al., 1992).

It is of interest to note that a cytotoxic effect was observed with ciprofloxacin-loaded PIBCA/NP when drug concentrations were above 8 μg/ml (equivalent to 40 μg of PIBCA per ml) and with blank PIBCA/NP used at the same dilution. A cytotoxicity with reduction of living cells after L929 fibroblast incubation in 20 μg/ml PIBCA/NP suspension has been already reported (Lherm et al., 1992). This could explain in our case the slight increase in the antimicrobial activity of ciprofloxacin when associated with nanoparticles. So, when ciprofloxacin-loaded PIBCA/NP was used at 4, 8, 16 and 32 μg/ml concentrations, those of PIBCA were 40, 80, 160 and 320 μg/ml, respectively. Therefore, PIBCA cytotoxicity could be greatly enhanced and the number of viable macrophages drastically reduced. In this case, it was somewhat normal that the total uptake of ciprofloxacin-loaded nanoparticles remained very low.

In conclusion, the association of ciprofloxacin with PIBCA nanoparticles led to significant modifications in the pharmacokinetic parameters after i.v. infusion to rabbits. Antimicrobial activity against MAC in human macrophages was in-

Table 2
Effect of ciprofloxacin in free form and loaded polyisobutylcyanoacrylate nanoparticles against MAC growth inside human macrophages

	Ciprofloxacin concentrations (μg/ml)							
	4		8		16		32	
	Day 4	Day 7	Day 4	Day 7	Day 4	Day 7	Day 4	Day 7
Ciprofloxacin (free form)	0	0	−0.65	−0.85	−0.68	−0.50	−0.80	−0.90
Ciprofloxacin-loaded PIBCA/NP	0	0	−1.10	−1.20	−1.30	−1.40	−1.80	−1.90

The listed values indicate the decrease of cfu count (log) observed at day 4 and day 7 compared with day 0.

creased from ciprofloxacin-loaded NP compared with solution. However efficiency remained much lower than expected due, at least in part, to the cytotoxicity of PIBCA. Unloaded PIBCA nanoparticles did not show any antimicrobial activity at any concentration.

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