Influence of Poly(DL-Lactide) Nanocapsules on the Biliary Clearance and Enterohepatic Circulation of Indomethacin in the Rabbit

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Received July 14, 1992; accepted September 28, 1992

Following intravenous administration, the uptake of colloidal drug carriers by cells of the mononuclear phagocyte system, mainly the Kuppfer cells, may concentrate an encapsulated drug close to the liver parenchymal cells and facilitate its biliary excretion and enterohepatic circulation. To test this hypothesis indomethacin was administered (10 mg/kg) in four groups of 10 rabbits each by intravenous infusion at a constant rate over 2 hr, either in its free form (aqueous solution) or as nanocapsules prepared from preformed poly(DL-lactide). Unchanged drug was assayed in plasma of the two control (sham-operated) groups and in both plasma and bile of the two bile-cannulated groups. Pharmacokinetic analysis led to the conclusion that the uptake of nanocapsules by liver macrophages reduces the concentration of the drug by enhancing its total clearance. This enhancement was due to an increase in biliary clearance, as a result of parallel increases in bile concentration and biliary excretion of the drug. It was also demonstrated that nanocapsules enhance the enterohepatic circulation of indomethacin.

KEY WORDS: nanocapsules; poly(DL-lactide); biliary clearance; enterohepatic circulation; indomethacin; rabbit.

INTRODUCTION

Following intravenous administration, colloidal drug carriers such as liposomes and polymeric nanoparticles are rapidly cleared from the blood and accumulate in liver, spleen, and bone marrow (1,2). These disposition kinetics are attributable mainly to the endocytic activity of cells of the mononuclear phagocyte system (3). The discontinuous endothelium of liver sinusoids and the abundance of Kuppfer cells render this organ a specific target for colloidal drug carriers. In metastasis-bearing mice, the uptake of doxorubicin-loaded nanoparticles by Kuppfer cells was shown to induce a reservoir effect and conditions for a favorable concentration gradient, thus increasing the delivery of the active drug from macrophages to the neighboring malignant cells of the hepatic parenchyma, with a better efficacy as compared to giving the drug as a solution (4). Moreover, polymeric vesicular drug carriers, i.e., nanocapsules prepared by interfacial polymerization of alkylcyanoacrylate monomer (5), were shown to be suitable for the delivery of immunostimulating drugs. Activation of the cytotoxicity of liver macrophages by nanocapsules loaded with muramyldipeptide-L-alanyl-cholesterol was successfully tested in the reticulosarcoma M 5076 model of hepatic metastasis (6).

We recently prepared nanocapsules from preformed, biocompatible, and biodegradable polymers, such as poly(lactide) (7). Following oral administration to the rat, the pharmacokinetic and pharmacological properties of indomethacin-loaded poly(DL-lactide) nanocapsules were shown to be similar to the properties of the free drug (8,9); however, gastrointestinal toxicity was dramatically reduced when indomethacin was administered in nanocapsules (10). Like many other acidic nonsteroidal antiinflammatory drugs [ibuprofen (11), piroxicam, and tenoxicam (12)], indomethacin exhibits extensive biliary excretion followed by intestinal reabsorption (13,14). The enterohepatic circulation of NSAIDs, and especially indomethacin, has been considered the primary causative factor for the gastrointestinal lesions following both oral and intravenous administration of these drugs (13). The oral administration of cholestyramine (15) or activated charcoal (16) reduced the enterohepatic circulation of the drug and enhanced its clearance. Thus, indomethacin can be used as a model drug to study the influence of the uptake of colloidal drug carriers by the liver on the biliary clearance and the enterohepatic circulation.

The aim of the present work was to study the biliary clearance and the enterohepatic circulation of indomethacin in rabbits following its intravenous infusion either in the free form (solution) or as drug-loaded nanocapsules prepared from preformed poly(DL-lactide).

MATERIALS AND METHODS

Chemicals

Indomethacin and benzylbenzoate were purchased from Sigma (St. Louis, MO); poly(DL-lactide) (Resomer R206; MW 109,000) was purchased from Boehringer-Ingelheim (Ingelheim, FRG). Phospholipid mixture (Epikuron 170) and poloxamer (Pluronic F68) were purchased from Lucas-Meyer (Hamburg, FRG) and ICI (Clamart, France), respectively. All other chemicals were of analytical grade and were purchased from Prolabo (Paris, France). Injectable indomethacin was the marketed product Indocid (MSD-Chibret, Paris, France).

Indomethacin-Loaded Nanocapsules

Nanocapsules were prepared according to the method already described (7). Briefly, a solution of indomethacin (100 mg), Resomer R206 (625 mg), benzylbenzoate (2.5 mL), and Epikuron 170 (625 mg) in acetone (125 mL) is added to an aqueous solution (250 mL) of Pluronic F68 (625 mg) under magnetic stirring. The resulting mixture turns milky instantaneously, because of the formation of nanocapsules by interfacial polymer deposition. Acetone is removed by rotor evaporation under reduced pressure; then the aqueous colloidal suspension is filtered through a sintered-glass filter

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0724-8741/93/0500-0750$07.00/0

(9–15 μm) and concentrated to 50 mL at 50°C. The drug concentration in the final suspension is 2 mg/mL.

The particle-size distribution of the resultant nanocapsules (220 ± 20 nm) was measured by light scattering using a monochromatic laser-ray diffusion counter (Super-Nanosizer N4, Coultronics, France). Drug payloads for nanocapsules and free drug in the aqueous suspending medium were measured following separation by a combined ultrafiltration-centrifugation technique (Centrisart I 20,000, Sartorius, FRG) and according to a modified HPLC technique (17). Indomethacin was shown to be completely encapsulated (>99%) using this loading technique, with no free drug detected in the aqueous suspending medium.

Animals

Experiments were carried out on fasted Fauve de Bourgogne male rabbits weighing 1.5–2.5 kg (Elevage de la Faurie, Cubjac, France). Ten animals were randomly assigned to each of six groups (A–F) as follows.

(A) Control rabbits (sham operated) received Indocid solution.
(B) Control rabbits (sham operated) received indomethacin as the nanocapsule suspension.
(C) Bile-cannulated rabbits received Indocid solution.
(D) Bile-cannulated rabbits received indomethacin as the nanocapsule suspension.
(E, F) Bile-cannulated rabbits (no treatment) were used as bile donors to rabbits in groups C and D, respectively.

Ethyl ether was used as anesthetic during bile cannulation and sham operation (total time, about 20 min). The treatment and the pharmacokinetic study were performed in conscious animals.

Treatment and Sampling Schedule

In order to avoid high, possibly toxic, initial concentrations following iv bolus injections, animals in groups A, B, C, and D received the 10 mg/kg indomethacin dose by intravenous infusion at a constant rate of 0.167 mL/min for 2 hr through a venous catheter implanted in one ear. Indocid solution and nanocapsule suspension were prepared by simple dilution with sterile saline and adjusted to the exact dose for each rabbit in a 20-mL infusion volume.

Blood samples (0.7 mL) were collected from groups A, B, C, and D in heparinized vials, through a venous catheter implanted in the other ear, at 0, 0.25, 0.5, 1, 1.5, 1.75, 2, 2.25, 2.5, 3, 4, 6, 8, 10, 12, and 24 hr after starting the infusion. Plasma was separated by centrifugation at 4°C and frozen prior to further processing. Bile was continuously collected during 24 hr from groups C, D, E, and F. Each animal in the E and F groups was paired with one rabbit in the C and D groups. The bile from the donor animal was directly transferred to the acceptor animal through a duodenal catheter. The bile from groups C and D was collected according to the following time intervals: 0–0.5, 0.5–1, 1–1.5, 1.5–2, 2–2.5, 2.5–3, 3–4, 4–5, 5–6, 6–7, 7–8, 8–9, 9–10, and 10–24 hr. The volume of each bile sample was exactly measured, then the samples were centrifuged at 4°C and the clear supernatant was frozen prior to further processing.

Preparation of Biological Sample

Plasma. Two hundred microliters of plasma was added to 200 μl of acetonitrile containing the internal standard (clomethacin, 6 μg/mL). Samples were mixed thoroughly and centrifuged. The clear supernatant was used for HPLC analysis.

Bile. One hundred microliters of bile, previously diluted (1/10) in blank bile when necessary, was mixed with 150 μL of 1 *M* hydrochloric acid and 100 μL of dichloroethane containing the internal standard (clomethacin, 10 μg/mL). Then, 2.9 mL of dichloroethane was added and mixed on a vortex mixer. After centrifugation, 2.25 mL of the organic extract was dried under nitrogen and the dry residue was dissolved in 300 μL of the mobile phase. Recovery values for indomethacin and clomethacin, evaluated by comparing extracted spiked samples with unextracted standard solutions, were 85 ± 3 and 64 ± 3%, respectively.

Assay Method for Indomethacin

Indomethacin concentrations in dosage forms, plasma samples, and bile samples were determined by HPLC using clomethacin as the internal standard (17). The HPLC equipment (Waters, Saint-Quentin en Yvelines, France) consisted of a Model 6000 A pump, a UV Lambda Max 480 detector, a chromatography work-station Baseline 810 and prepacked Microbondapack C_{18} columns. The mobile phase was a mixture of acetonitrile and 0.25 m*M* sodium acetate (55:45, v/v), the pH being adjusted to 3.5 with acetic acid. The flow rate was 1.5 mL/min and detection was performed at 254 nm. In these conditions, the retention times were 7.4 min for indomethacin and 5.3 min for the internal standard. The detection limit for indomethacin was 0.05 μg/mL. The standard curve (indomethacin/clomethacin peak area ratio) was linear between 0.05 and 48.0 μg/mL (r = 0.992).

Pharmacokinetic Analysis

The area under the curve (AUC) of indomethacin concentrations (plasma and bile) was calculated following the trapezoidal rule during the experimental period (AUC[0–t]). The extrapolated area (AUC[t–∞]) was calculated by dividing the last experimental concentration (C_t) by the rate constant (K) of the terminal phase. The total area (AUC$^{\infty}$) was obtained by adding the experimental area to the extrapolated area:

$$\mathrm{AUC}^{\infty} = \mathrm{AUC}[0\text{–}t] + \mathrm{AUC}[t\text{–}\infty] \quad (1)$$

$$\mathrm{AUC}[t\text{–}\infty] = C_t/K \quad (2)$$

The half-lives were calculated from the rate constant (K) corresponding to the log-linear decrease in terminal plasma or bile concentrations and biliary excretion rates:

$$t_{1/2} = 0.693/K \quad (3)$$

The total plasma clearance (CI_T) was calculated from the equation

$$CI_T = \text{Dose}/AUC^{\infty} \quad (4)$$

The biliary clearance (CI_B) was calculated from the equation:

$$CI_B = f_B \cdot CI_T \quad (5)$$

where f_B is the fraction of dose excreted in 24 hr as unchanged drug by the biliary route, assuming total excretion during this period of time.

The volume of distribution was calculated from the equation

$$V_D = CI_T/K \quad (6)$$

Statistical Analysis

All data presented in the tables are mean (±SD) values. They were analyzed for significant differences using the Student *t* test. A *P* value less than 0.05 was considered to be statistically significant.

RESULTS

Plasma Data

Comparative time profiles of mean (±SD) indomethacin plasma concentrations in rabbits receiving the drug as a solution and as nanocapsules are illustrated in Figs. 1 and 2, respectively. Plasma pharmacokinetic parameters derived from experimental data in control and in bile-cannulated rabbits are presented in Table I and II, respectively.

Control Rabbits. Rabbits receiving nanocapsules exhibited lower concentrations at the end of the infusion period (6.7 ± 1.4 μg/mL) than rabbits receiving the free drug (13.6 ± 3.5 μg/mL) ($P < 0.001$). Control rabbits receiving nanocapsules also exhibited a secondary peak during the terminal phase (cf. example of rabbit B4 illustrated in Fig. 3), whereas this secondary peak was less marked, or absent, in animals receiving the solution. In comparison to the volume of distribution after administration of the solution (1.6 ± 0.6 L/kg), a significant increase was observed after dosing of nanocapsules (3.2 ± 1.9 L/kg) ($P < 0.05$), while the total clearance

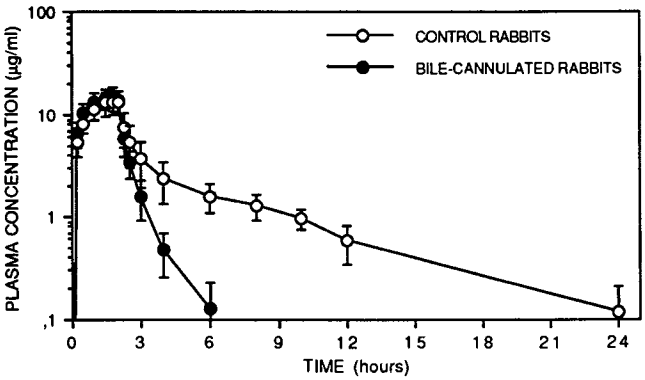

Fig. 1. Mean (±SD) plasma concentration (μg/mL) of indomethacin in rabbits following a 10 mg/kg intravenous infusion over 2 hr as a solution.

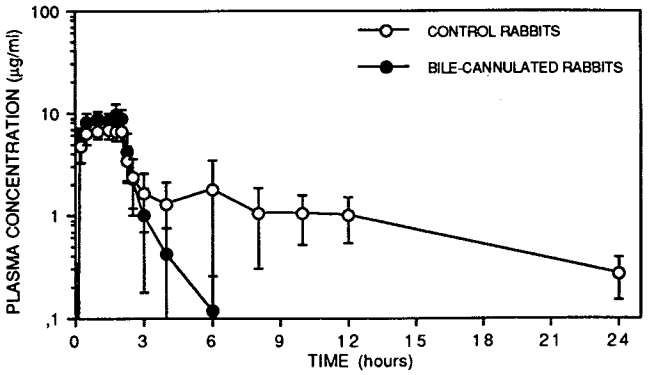

Fig. 2. Mean (±SD) plasma concentration (μg/mL) of indomethacin in rabbits following a 10 mg/kg intravenous infusion over 2 hr as nanocapsules.

following nanocapsules was slightly larger (4.9 ± 1.6 mL/min · kg) than the clearance of the free drug (3.8 ± 0.6 mL/min · kg) (NS). Nanocapsules significantly increased the half-life, 7.2 ± 2.9 hr, compared to the 4.8 ± 1.7 hr following the free drug ($P < 0.05$).

Bile-Cannulated Rabbits. As in noncannulated rabbits, nanocapsules had a lowered concentration at the end of the infusion period (8.9 ± 1.8 μg/mL) compared to the solution (13.8 ± 3.3 μg/mL) ($P < 0.001$). The volume of distribution was slightly larger following nanocapsules (0.83 ± 0.39 L/kg) than after the solution (0.53 ± 0.25 L/kg) (NS). Secondary peaks were not observed with either dosage form, and half-lives were in the same range with the solution and nanocapsules (1.1 ± 0.6 and 1.2 ± 0.6 hr, respectively) (NS). Nanocapsules also significantly increased the total clearance, 8.4 ± 1.6 mL/min · kg, compared to the solution, 5.8 ± 1.2 mL/min · kg ($P < 0.001$).

Control vs Bile-Cannulated Rabbits. Additional statistical data comparing pharmacokinetic parameters between control and bile-cannulated groups receiving the same dosage form are presented in Table III. Whatever the dosage form, the total clearance of indomethacin in bile-cannulated rabbits was increased compared with control ($P < 0.001$ for the solution and nanocapsules). Larger volumes of distribution ($P < 0.001$) and longer terminal half-lives ($P < 0.001$) were also observed with both dosage forms in control rabbits.

Bile Data

Mean (±SD) bile flows are illustrated in Fig. 4. Parmacokinetic parameters derived from bile-data are presented in Table IV.

While nanocapsules decreased plasma concentrations, they led to higher bile concentrations at the end of the infusion period (0.81 ± 0.43 mg/mL) than did the solution (0.38 ± 0.26 mg/mL) ($P < 0.05$). Bile concentration profiles were biphasic with both dosage forms and exhibited similar slopes of terminal phases. Concentrations were still detectable at 24 hr: 3.5 ± 2.0 μg/mL (solution) and 8.3 ± 6.3 μg/mL (nanocapsules) ($P < 0.05$). Indomethacin bile concentrations were much higher than the corresponding plasma concentrations, with a bile/plasma ratio at the end of the infusion period of 29 ± 20 for the solution and 87 ± 37 for nanocapsules ($P <$

Table I. Pharmacokinetic Parameters (Plasma) of Indomethacin in Control Rabbits Following an Intravenous Infusion of 10 mg/kg over 2 hr[a]

	Solution	Nanocapsules	P
C_2 (μg/mL)	13.6 ± 3.5 (9.6–20.1)	6.7 ± 1.4 (4.8–8.7)	<0.001
C_{24} (μg/mL)	0.12 ± 0.09 (ULQ–0.28)	0.27 ± 0.12 (0.14–0.50)	<0.05
AUC^{∞} (μg · hr/mL)	46 ± 10 (37–70)	37 ± 10 (20–50)	NS
Cl_T (mL/min · kg)	3.8 ± 0.6 (2.4–4.6)	4.9 ± 1.6 (3.3–8.5)	NS
$t_{1/2}$ (hr)	4.8 ± 1.7 (2.9–8.8)	7.2 ± 2.9 (4.7–15.1)	<0.05
V_D (L/kg)	1.6 ± 0.6 (0.8–2.7)	3.2 ± 1.9 (1.8–7.5)	<0.05

[a] C_2, concentration at the end of the infusion period; C_{24}, concentration at the end of the experimental period (24 hr); AUC^{∞}, total area under the curve; Cl_T, total clearance; $t_{1/2}$, terminal half-life; V_D, volume of distribution; ULQ, under the limit of quantification; NS, not significant.

0.001). By taking into account the corresponding AUCs, these ratio increased to 40 ± 25 for the solution and 129 ± 45 for nanocapsules ($P < 0.001$).

Mean (±SD) biliary excretion rates are presented in Fig. 5. In contrast to the corresponding plasma concentrations, biliary excretion rates were larger in rabbits dosed with nanocapsules. Slow elimination phases were observed following both dosage forms, with similar, but highly variable, half-lives: 9.7 ± 9.1 and 8.4 ± 2.9 hr for the solution and nanocapsules, respectively. The cumulative biliary excretion was 3.5 ± 1.0 mg (18 ± 5% of the dose) following the free drug, while nanocapsules doubled this value to 7.4 ± 1.9 mg (35 ± 10% of the dose) ($P < 0.001$). A marked enhancement of the biliary clearance was shown following nanocapsules (2.9 ± 0.8 mL/min · kg), corresponding to almost three times the value observed with the solution (1.0 ± 0.4 mL/min · kg) ($P < 0.001$).

DISCUSSION

Plasma Pharmacokinetics in Control Rabbits

Secondary peaks may reflect the enterohepatic circulation of indomethacin and were already observed following the administration of free indomethacin in rats (18), in human volunteers (19), and in beagle dogs (20). Here, the higher secondary peaks induced by nanocapsules seem to be due to an accumulation of the carrier in the liver, thus increasing both the biliary excretion and the intestinal reabsorption (cf. bile data). Colloidal carriers themselves do not appear to cause enterohepatic cycling-based secondary peaks. For example, when vincamine (a poorly enterohepatic-cycled drug) was formulated as nanoparticles, no secondary peaks were observed (21). A larger volume of distribution was shown here in rabbits receiving nanocapsules. Similar results were observed in rats receiving indomethacin-loaded nanocapsules (18) and in rabbits after an intravenous bolus of vincamine-loaded nanoparticles (21). Thus, the uptake of colloidal carriers by the Kuppfer cells seems to introduce an added compartment of distribution, the liver, whereas this organ is generally included in the central (eliminating) compartment because of its high blood flow and its major role in the elimination of drugs. As already observed in rats following an intravenous injection of indomethacin-loaded nanocapsules (18), the colloidal carrier appears to increase also the half-life of the drug. However, the total clearance following nanocapsules was not significantly larger than the clearance of the free drug. Thus, the increase in the volume should be compensated for, at least partially, by a parallel increase in the half-life. Similar results have been observed in rabbits after an intravenous injection of vincamine-loaded nanoparticles (21).

The similar behavior of the vincamine-loaded carrier compared with the indomethacin-loaded carrier could not be attributed to the carriers rather than to the drugs. Whatever the processes involved (excretion of the unchanged drug or metabolism), elimination may occur only with the free drug, implying its *in vivo* release from the carrier. A previous study (10) has shown that indomethacin release from nanocapsules depends on its partition coefficient between the core of nanocapsules and their aqueous environment. This release is practically instantaneous when favorable conditions are met in the aqueous phase, such as a physiological pH (allowing rapid dissolution of the drug) and the presence of proteins (on which indomethacin can bind). On the other hand, as vincamine was superficially adsorbed onto (but not

Table II. Pharmacokinetic Parameters (Plasma) of Indomethacin in Bile-Cannulated Rabbits Following an Intravenous Infusion of 10 mg/kg over 2 hr[a]

	Solution	Nanocapsules	P
C_2 (μg/mL)	13.8 ± 3.3 (9.6–18.9)	8.9 ± 1.8 (6.9–12.8)	<0.001
AUC^{∞} (μg · hr/mL)	30 ± 6 (20–42)	21 ± 5 (16–32)	<0.001
Cl_T (mL/min · kg)	5.8 ± 1.2 (3.9–8.3)	8.4 ± 1.6 (5.2–10.2)	<0.001
$t_{1/2}$ (hr)	1.1 ± 0.6 (0.4–2.6)	1.2 ± 0.6 (0.3–2.0)	NS
V_D (L/kg)	0.53 ± 0.25 (0.20–0.97)	0.83 ± 0.39 (0.21–1.30)	NS

[a] C_2, concentration at the end of the infusion period; AUC^{∞}, total area under the curve; Cl_T, total clearance; $t_{1/2}$, terminal half-life; V_D, volume of distribution; NS, not significant.

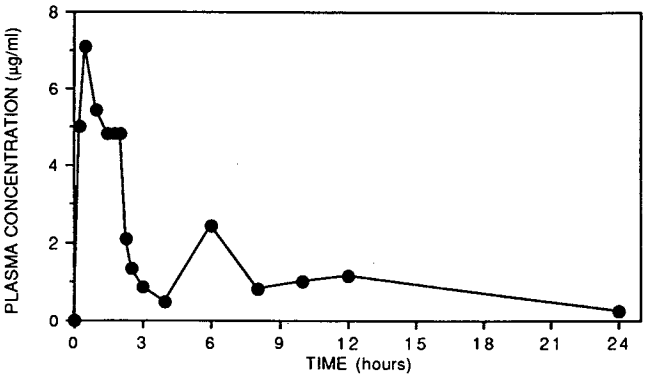

Fig. 3. Indomethacin plasma concentration (µg/mL) in control rabbit B4 dosed with indomethacin-loaded nanocapsules, illustrating the enterohepatic circulation (secondary peak).

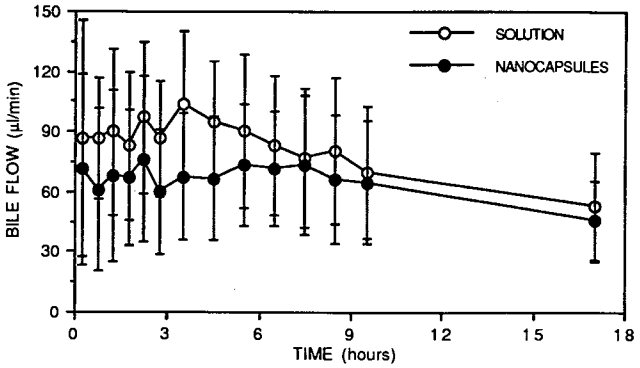

Fig. 4. Mean (±SD) bile flow (µL/min) in bile-cannulated rabbits following an intravenous infusion of indomethacin (10 mg/kg) over 2 hr.

embedded within) the matrix of nanoparticles (18), its *in vivo* release should also have been very rapid. Thus, the drug carriers did not seem to act as sustained-release systems, the two drugs being rapidly available in the free form to be eliminated following their own usual pathways.

The pharmacokinetic parameters in control rabbits receiving the solution were different here from the one found in other studies. As an example, the total clearance was about 3.8 mL/min · kg in our study, but only 1.9 mL/min · kg in both studies by Al-Meshal *et al.* (15) and El-Sayed *et al.* (16). However, here the total clearance was close to the one measured (3.6 mL/min · kg) in the historic study by Duggan *et al.* (13). This apparent agreement is not relevant, as Duggan *et al.* used the sum of free and conjugated unchanged drug plus metabolites for the calculation of clearance values. These discrepancies could be due to the longer duration in our study (24 vs 6 hr in the three mentionned references), thus allowing more accurate description and pharmacokinetic calculations.

Plasma Pharmacokinetics in Bile-Cannulated Rabbits

The most noticeable effect of bile cannulation is a marked increase in the apparent elimination of the drug, reflected by the rapid and monophasic decline of concentrations compared with the biphasic decline observed in the control groups receiving the same dosage form. The slope of the decline in bile-cannulated rabbits appears very close to the slope of the distribution phase in control groups. Secondary peaks were not observed following either dosage form in bile-cannulated rabbits, confirming the major role of enterohepatic circulation in this phenomenon. As already observed in the corresponding control group, bile-cannulated rabbits dosed with nanocapsules exhibited lower concentration at the end of the infusion period, but nanocapsules did not enhance significantly the volume of distribution.

In contradiction with the stability of the total clearance in control rabbits with dosage forms, nanocapsules led to a significant increase in the clearance of bile-cannulated rabbits. This result suggests that nanocapsules could markedly enhance a specific clearance mechanism especially sensitive to the targetting of the liver in bile-cannulated rabbits, but absent in control rabbits. Thus, the uptake of nanocapsules by the Kuppfer cells seems not only to introduce an added compartment of distribution, the liver, but also to render it a more efficient organ of elimination. The liver specificity of this clearance mechanism could be attributable to either a metabolic or an excretion process. As the phenomenon was observed only in bile-cannulated rabbits, thus precluding any enterohepatic circulation of indomethacin metabolites, biliary rather than metabolic clearance of indomethacin appears to be involved.

Table III. Statistical Comparison of Indomethacin Pharmacokinetic Parameters (Plasma) Between Control and Bile-Cannulated Rabbits Following an Intravenous Infusion of 10 mg/kg over 2 hr[a]

	Solution	Nanocapsules
C_2 (µg/mL)	NS	<0.01
AUC^{∞} (µg · hr/mL)	<0.001	<0.001
Cl_T (mL/min · kg)	<0.001	<0.001
$t_{1/2}$ (hr)	<0.001	<0.001
V_D (L)	<0.001	<0.001

[a] C_2, concentration at the end of the infusion period; AUC^{∞}, total area under the curve; Cl_T, total clearance; $t_{1/2}$, terminal half-life; V_D, volume of distribution; NS, not significant.

Control vs Bile-Cannulated Rabbit Plasma Comparisons

Whatever the dosage form used, the total clearance in bile-cannulated rabbits was markedly increased when compared to control groups. This result is related to the suppression of the intestinal reabsorption following the cannulation of the bile duct and confirms the major influence of the enterohepatic circulation on the disposition kinetics of indomethacin. Comparison of the AUCs shows that the enterohepatic circulation may enhance the systemic exposure to the drug by about 50% when indomethacin is administered as a solution and by nearly 80% when nanocapsules are used. Intestinal reabsorption is also related to the prolonged terminal half-life observed in control rabbits, while its absence in bile-cannulated rabbits reveals the true elimination rate of the drug. The volume of distribution was markedly larger in control rabbits, thus illustrating that the enterohepatic circulation is a distribution, and not an elimination, process.

Table IV. Pharmacokinetic Parameters (Bile) of Indomethacin in Bile-Cannulated Rabbits Following an Intravenous Infusion of 10 mg/kg over 2 hr[a]

	Solution	Nanocapsules	P
Bile vol. [0–24 hr] (mL)	96 ± 36 (38–165)	79 ± 31 (25–121)	NS
C_2 bile (mg/mL)	0.38 ± 0.26 (0.15–0.99)	0.81 ± 0.43 (0.26–1.49)	<0.05
C_{24} bile (μg/mL)	3.5 ± 2.0 (0.01–5.6)	8.3 ± 6.3 (3.5–21.0)	<0.05
A_B [0–24 hr] (mg)	3.5 ± 1.0 (2.1–5.7)	7.4 ± 1.9 (4.1–10.6)	<0.001
f_B (% dose)	18 ± 5 (10–26)	35 ± 10 (16–52)	<0.001
Cl_B (mL/min · kg)	1.0 ± 0.4 (0.5–1.7)	2.9 ± 0.8 (1.6–4.1)	<0.001
$t_{1/2}$ exc rate (hr)	9.7 ± 9.1 (1.9–26.6)	8.4 ± 2.9 (2.1–13.9)	NS

[a] Bile vol. [0–24 hr], cumulative volume secreted in 24 hr; C_2, concentration at the end of the infusion period; C_{24}, concentration at the end of the experimental period (24 hr); A_B[0–24 hr], cumulative biliary excretion in 24 hr; f_B, percentage of the dose excreted in 24 hr; Cl_B, biliary clearance; $t_{1/2}$ exc rate, terminal half-life calculated from the biliary excretion rate of the drug; NS, not significant.

Bile Pharmacokinetics

It is clear from the bile flow data in Fig. 4 and from the cumulative volumes in Table IV that dosage forms had little or no influence on the rate of bile secretion. However, nanocapsules markedly enhanced both bile concentrations and biliary excretion rates. Bile concentration curves and biliary excretion rate profiles were biphasic, with a slow terminal phase which was not seen in the corresponding plasma concentration curves, probably because of the lack of sensitivity of the assay method. Most of the biliary elimination occurs rapidly (90% in 4 hr, including the infusion period) in both groups of bile-cannulated rabbits, thus limiting the practical interest of this terminal phase. The increase in the biliary excretion and the decrease in the plasma AUCs following nanocapsules contribute to the marked enhancement of the biliary clearance as compared to the solution. The accumulation of nanocapsules in Kuppfer cells, leading to high concentrations in the liver (4), may explain this result. The plot of cumulative biliary excretion versus cumulative plasma AUC in bile-cannulated rabbits is illustrated in Fig. 6. The slope of this plot, i.e., the biliary clearance, is markedly increased by nanocapsules compared with the solution, thus confirming the data presented in Table IV. It can also be seen from Fig. 6 that the biliary clearance of indomethacin is not constant and tends to increase with time.

The discrepancy between the total clearance here and the literature data has already been discussed (cf. control rabbits dosed with the solution). The same findings apply to the biliary clearance: 1 mL/min · kg with the solution in the present study, while Duggan *et al.* (13) obtained 0.4 mL/min · kg by using data (sum of free plus conjugated drug and metabolites) leading to higher clearance values than by using unchanged drug data. As mentioned previously, we think that the experimental period (24 hr in the present study but only 6 hr in the study by Duggan *et al.*) should lead to a more accurate evaluation of the given parameter.

Bile/plasma ratios of concentrations and corresponding AUCs demonstrate the capacity of the liver to concentrate the drug in the bile. By taking into account the high binding of indomethacin to plasma proteins, these results strongly confirm an active extraction from the plasma and concentration of the drug in the bile by hepatocytes. The higher ratios observed with nanocapsules confirm the major role of their uptake by the Kuppfer cells; this uptake leads to a rapid extraction of the drug-loaded carrier from the plasma and to their high concentrations in liver macrophages, resulting in a high gradient close to hepatocytes and favoring the biliary excretion of the drug.

In conclusion, the results of the present study demonstrate that nanocapsules, prepared from poly(DL-lactide), reduce indomethacin concentrations following an intravenous administration of this drug by enhancing its total clearance in both control and bile-cannulated rabbits. Nanoencapsulation

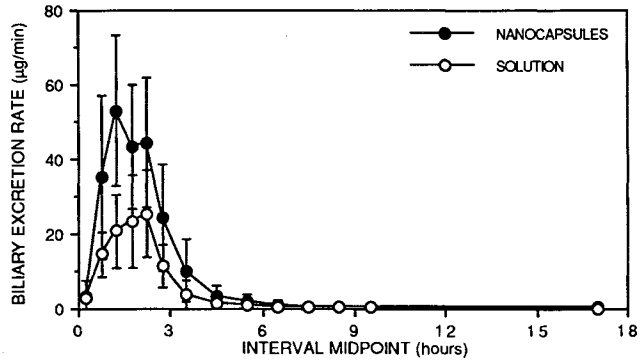

Fig. 5. Mean (±SD) biliary excretion rate (μg/min) of indomethacin following the intravenous infusion of 10 mg/kg over 2 hr in bile-cannulated rabbits.

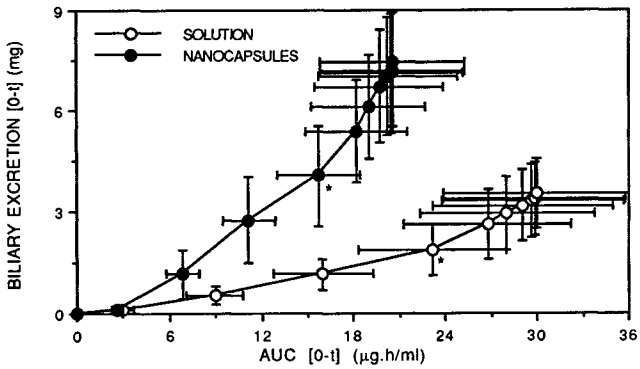

Fig. 6. Plot of the mean (±SD) cumulative biliary excretion of indomethacin (μg) vs the mean (±SD) cumulative area under plasma concentrations (μg · hr/mL) following the intravenous infusion of 10 mg/kg over 2 hr in bile-cannulated rabbits. (*) End of the infusion.

enhances indomethacin concentration in the bile, thus resulting in a marked increase in both the biliary clearance and the enterohepatic circulation of the drug.

Enterohepatic circulation of acidic NSAIDs, such as indomethacin, has been considered a primary causative factor for the gastrointestinal lesions observed following both their oral and their systemic administration. If enterohepatic circulation of indomethacin were such a major factor, one would expect to find more lesions after administration of the encapsulated drug. However, we have recently shown that indomethacin-loaded nanocapsules protected gastrointestinal mucosae from the ulcerative effect of the drug in rats, following its repeated oral administration at doses as large as 10 mg/kg during 3 consecutive days (10). Thus, while the previous assumption is consistent with the bile data obtained in the present study following an intravenous administration, the tolerance observed after repeated oral administration supports the hypothesis that direct contact of indomethacin with the gastrointestinal mucosae should be a determinant factor. A comparative study of the gastrointestinal toxicity of intravenously administered indomethacin as a solution and nanocapsules will help us to confirm these findings.

ACKNOWLEDGMENTS

This work was partly supported by the Conseil Régional d'Aquitaine and Ministère de l'Education Nationale of France.

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