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Disposition and protective effect against irritation after intravenous and rectal administration of indomethacin loaded nanocapsules to rabbits

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Abstract

Indomethacin loaded poly(D,L-lactide) nanocapsules (PLA-NCs) were investigated after intravenous and rectal administration to rabbits. A rebound of indomethacin plasma concentrations attributed to enterohepatic circulation of indomethacin was observed with all preparations. Following i.v. infusions, results showed that PLA-NCs altered the pharmacokinetics of indomethacin in ways that accelerate the extravascular distribution by enhancing the capture of the colloidal carrier by the liver and, at the same time, modifying the elimination rate of indomethacin. After rectal administration of indomethacin formulations, drug plasma concentration profiles revealed that absorption was more complete and more progressive with nanocapsules than with solution. T_{max} had nearly the same value for all formulations, and bioavailability of indomethacin by this route was increased by nanoencapsulation. The terminal half-life of indomethacin was significantly lower when the drug was given by the rectal route either in solution by PLA-NCs, as compared to suppository. PLA-NCs exhibited a protective effect against the rectal irritability of indomethacin which can be attributed to the reduction of direct contact of free indomethacin with rectal mucosa.

Keywords: Biodegradable nanocapsules; Indomethacin; Pharmacokinetics; Poly(D,L-lactide); Rectal irritation

1. Introduction

Many colloidal polymeric drug delivery systems have been developed and investigated over the last few decades. Some of them are of great therapeutic interest owing to their ability to improve the oral bioavailability of drugs (Andrieu et al., 1989; Chiannilkulchai et al., 1990; Maincent et al., 1984, 1986), to protect the gastro-intestinal mucosa from irritation after oral administration (Ammoury et al., 1989; Andrieu et al., 1986), to significantly modify the tissue distribution of

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drugs (Allemann et al., 1994; Bapat and Boroujerdi, 1993a,b; Chiannilkulchai et al., 1990; Couvreur et al., 1980; Gipps et al., 1988; Illum et al., 1984; Kreuter et al., 1979), or finally, to increase the therapeutic activity of drugs after intravenous infusion (Youssef et al., 1988). However, most in vivo investigations, have focused on the disposition and therapeutic interest of colloidal systems after administration by parenteral and oral routes (Ammoury et al., 1991; Andrieu et al., 1986, 1989; Damgé et al., 1987; Soehngen et al., 1988). To our knowledge, the disposition and protective effects of colloidal polymeric drug carrier systems on rectal mucosa have never been investigated after rectal administration.

The aim of this work was to study the influence of poly(D,L-lactide) nanocapsules (PLA-NCs) and the route of administration (intravenous and rectal) on indomethacin pharmacokinetics using the rabbit as animal model. Moreover, part of this investigation was devoted to evaluate the ability of PLA-NCs to protect the rectal mucosa against the ulcerating effects of indomethacin given in various formulations by the rectal route.

2. Materials and methods

2.1. Materials

Indomethacin and benzylbenzoate were obtained from Sigma Ltd. (St. Louis, MO), poly(D,L-lactide). Resomer® R206, mol. wt. 110 000 was purchased from Boehringer Ingelheim (Germany), phospholipids (Epikuron® 170) and poloxamer 188 (Pluronic® F68) were furnished respectively by Lucas Meyer (Hamburg, Germany) and ICI (Clamart, France). All other components and solvents were of analytical grade and purchased from Prolabo (France).

2.2. Sample preparation

2.2.1. Nanocapsule suspensions

The indomethacin loaded poly(D,L-lactide) nanocapsules were prepared according to the Fessi et al. (1989) procedure. Briefly, an acetone solution containing poly(D,L-lactide), indomethacin, benzylbenzoate and phospholipids was added, under moderate magnetic stirring, to an aqueous solution of poloxamer 188. Nanocapsules were formed instantaneously by interfacial polymer deposition. Acetone was removed and the colloidal suspension was concentrated by rotor evaporation under vacuum and filtered through sintered glass (9–15 μm). In its final form the prepared nanocapsule suspension had the following composition: indomethacin (0.250 g), benzylbenzoate (10 ml), poly(D,L-lactide) (2.50 g), phospholipids (2.50 g), poloxamer 188 (2.50 g) and deionized water (up to 100 ml). The drug payload in the aqueous suspending medium was assayed according to the HPLC modified Drouet et al. (1981) method, as described below in the pharmacokinetic study. The aqueous suspension medium was previously separated using a centrifugal ultrafiltration technique. It was found that all the initial amount of indomethacin was nanoencapsulated. Afterwards, this suspension was diluted with water for injection in order to administer the calculated dose of indomethacin in a final volume of 10 or 20 ml.

Another indomethacin free PLA-NC suspension was also prepared under the same conditions as described above, but omitting drug. The final pH of both colloidal suspensions was between 3.50 and 4.50. The mean size of the nanocapsules as measured by a laser-light scattering system (Super-Nanosizer, Coultronics, France) was of 220 $\pm$ 20 nm.

2.2.2. Indomethacin solutions and suppositories

Indomethacin aqueous solutions for intravenous and rectal administration were prepared by extemporaneous reconstitution of INDOCID® injection (MSD and CHIBRET, France) using water for injection.

The indomethacin suppositories were obtained by drawing up melted and homogenized suppositories of INDOCID® 50 mg (MSD and CHIBRET, France), without any modification of its composition, into the cut tip of a 1-ml syringe. This procedure allowed adjustment of the amount of suppository to be administered according to the animal's weight, and facilitated insertion of the suppository into the animal's rectum.

2.3. Other formulations

In order to complete the protection effect study, three other formulations were prepared: normal saline solution (USP XXII), aqueous emulsion of benzylbenzoate (10 ml of benzylbenzoate were dispersed in 40 ml of 0.25 mg/ml poloxamer 188 aqueous solution for 15 min at 3000 rev./min), and aqueous emulsion of indomethacin/benzylbenzoate (10 ml of benzylbenzoate containing 250 mg of indomethacin were dispersed in 40 ml of 0.25 g/ml poloxamer aqueous solution for 15 min at 3000 rev./min).

2.4. Animals

Experiments were carried out on healthy adult male rabbits 'Fauve de Bourgogne' weighing between 2.2 and 2.5 kg (Elevage de la Faurie, Cubjac, France). The animals were fasted for 18 h prior to and 24 h following treatment but had free access to tap water. Two groups of rabbits (n = 10) received indomethacin formulations by intravenous infusion. Nine other groups of rabbits (n = 6) were given nine formulations (one formulation each group) by the rectal route.

2.5. Pharmacokinetic study

2.5.1. Administration of samples

2.5.1.1. Dose. Whatever the indomethacin formulation or administration route used, a single dose of 10 mg drug per kg of body weight was given to each animal.

2.5.1.2. Intravenous infusions. Indomethacin solution and indomethacin loaded PLA-NC suspension, containing the calculated drug dose in a final volume of 20 ml, were administered by intravenous infusion (Harvard '11' Syringe Pump, EALING, Les Ulis, France) at a constant rate over 2 h via the marginal ear vein.

2.5.1.3. Rectal administration. Five indomethacin formulations were given by rectal route: aqueous solutions (A and B) containing the calculated dose administered in a final volume of 1 and 10 ml, respectively, nanocapsule suspensions (A and B) containing the calculated dose in a final volume of 10 and 20 ml, respectively and suppository. The liquid preparations were administered through a rectal cannula. In all cases, after administration, the rectum of the rabbit was clamped with a clip in order to prevent leakage of the product.

2.5.2. Determination of indomethacin plasma levels

After administration of drug containing preparations, blood samples (0.5 ml) were taken at defined times up to 24 h, using heparinized tubes, from all animals by marginal ear vein puncture. Plasma was isolated by centrifugation (3500 rev./min), frozen and stored until assayed for indomethacin. Drug concentrations in plasma were determined by employing a modified HPLC method (Drouet et al., 1981) and using clomethacin as internal standard. The HPLC equipment (Waters, Saint-Quentin en Yvelines, France) consisted of a Model 6000 A pump, a model Wisp 712 auto-sampler, a UV Lambda Max 480 detector and a Data Module 730 integrator. Separation was achieved at room temperature on a prepacked Microbondapack C18 column. The mobile phase was a (55:45, v/v) mixture of acetonitrile and 0.25 mM sodium acetate. The pH was 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 time was 7.4 min for unmetabolized indomethacin and 5.3 min for clomethacin.

2.5.3. Pharmacokinetic and statistical analyses of the data

C_{max} and T_{max} were extracted from intravenous and rectal data. The half-life ($t_{1/2}$) was calculated from the terminal phase of the elimination curves. Total area under the curves ($AUC_{0-\infty}$) was calculated using the trapezoidal rule with extrapolation to infinity according to equation:

$$AUC_{24-\infty} \frac{C_P}{K_e}$$

where C_p is the indomethacin plasma concentration at 24 h and K_e the elimination rate constant. The total body clearance (Cl) and the apparent volume of distribution (V_d) were calculated as:

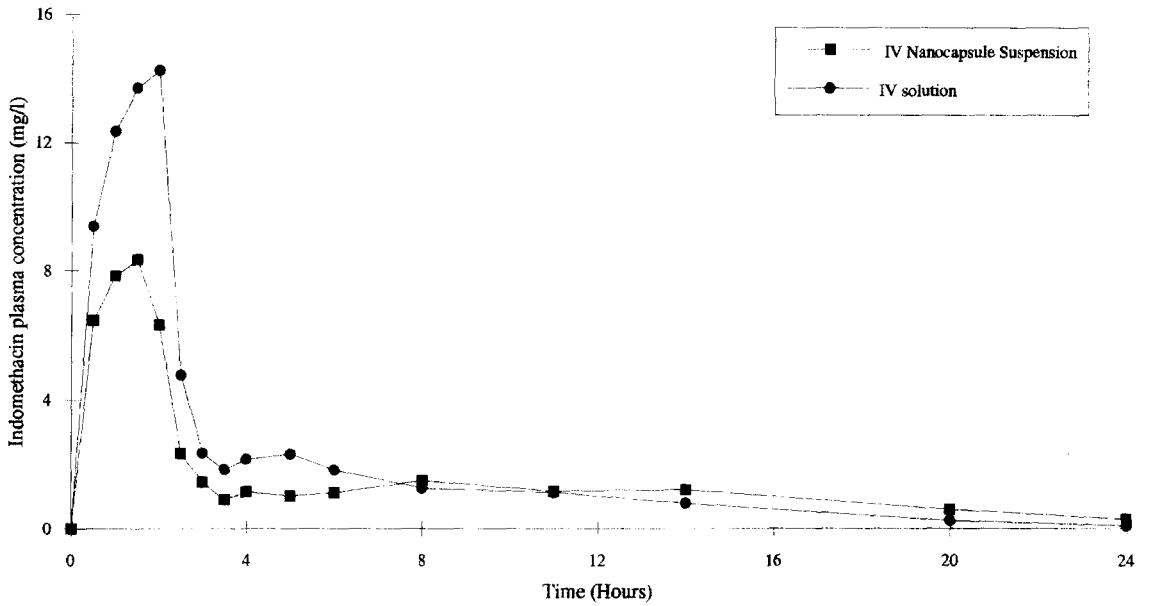

Fig. 1. Mean plasma concentration-time profiles of indomethacin following intravenous infusion of indomethacin solution and loaded poly(D,L-lactide) nanocapsules at a dose of 10 mg drug/kg to rabbits.

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

$$V_d = \frac{Cl}{K_e}$$

All data were expressed as mean value ± S.D. Statistical analysis was performed using Student's t-test. Mean differences were considered significant at level $P < 0.05$.

2.6. Protective effect study

This investigation was carried out on all animals used in the pharmacokinetic study and to which indomethacin formulations were given either by intravenous or by rectal route. Furthermore, in order to explore the potential irritant effect on the rectal mucosa of some components used in preparing indomethacin loaded PLA-NCs, four other groups of rabbits ($n = 6$) received the following formulations (one formulation per group) by the rectal route, under the same conditions as in the pharmacokinetic study: normal saline solution (control group), suspension of indomethacin free PLA-NCs, aqueous emulsion of benzylbenzoate, and aqueous emulsion of indomethacin–benzylbenzoate. Doses to be administered were calculated supposing that each formulation contained 2.5 mg of indomethacin per ml and on the basis of the theoretical dose of 10 mg of indomethacin/kg. Each dose was then diluted up to 10 ml with water before administration. All animals used (74 rabbits) were killed 24 h following rectal administration. Rectum and distal colon were excised, slit open and rinsed carefully with normal saline solution. The mucosal surface was then examined for detection of superficial macroscopic lesions using a dissecting binocular microscope.

3. Results and discussion

3.1. Presentation

The mean plasma concentration-time profiles after intravenous infusion of the aqueous solution and the PLA-NCs containing indomethacin are presented in Fig. 1. Fig. 2 presents the profiles obtained after rectal administration of five indomethacin formulations: aqueous solutions A

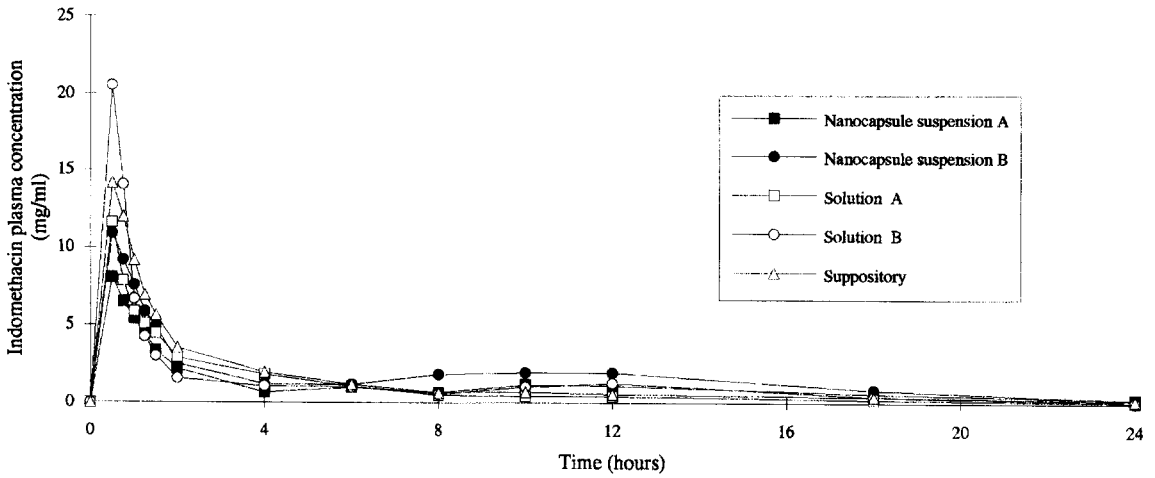

Fig. 2. Mean plasma concentration-time profiles of indomethacin after rectal administration of five indomethacin formulations: solutions A and B, poly(D,L-lactide) nanocapsule suspensions A and B and suppository at a dose of 10 mg/kg to rabbits.

and B, PLA-NC suspensions A and B and suppository. Pharmacokinetic parameters (means ± S.D.) obtained after intravenous and rectal administration of indomethacin formulations are reported in Table 1.

3.2. Pharmacokinetic study

3.2.1. Intravenous administration

Indomethacin plasma concentrations between 0 and 3.5 h following i.v. infusion were lower with nanocapsule suspension than with solution. This phenomenon has already been described by many authors (Couvreur et al., 1980, Grislain et al., 1983) and may be attributed to the uptake of nanocapsules by Kupffer cells. With both formulations (solution and nanocapsule suspension), a plasma concentration rebound was observed 2 h after infusion was stopped. This result could be explained by the enterohepatic circulation of indomethacin.

Mean maximum plasma indomethacin concentration (C_{max}) was measured for the indomethacin solution and nanocapsule suspension at 2 h and 1.30 ± 0.20 h, respectively after the beginning of the intravenous infusion. At the end of the administration, mean indomethacin plasma concentration with PLA-nanocapsules was lower than the mean C_{max} value. This phenomenon was also observed in all animals to whom the indomethacin PLA-NC suspension was given. We do not have a clear explanation of this result, but it was probably due to the uptake of a very large quantity of nanocapsules by the reticuloendothelial tissues.

After i.v. infusion of indomethacin nanocapsules, the mean C_{max} value (9.2 ± 2.2 mg/l) was lower than that obtained with solution (14.2 ± 3.6 mg/l) and was followed by a more rapid decrease of drug plasma levels indicating that the extravascular distribution of indomethacin was accelerated. This result was confirmed by the dramatic increase in the apparent volume of distribution (V_d) of the drug with nanocapsules (2360 ± 580 ml/kg) as compared to solution (920 ± 253 ml/kg). On the other hand, the terminal half-life of indomethacin ($t_{1/2}$) was increased by nanoencapsulation.

Since the total plasma clearance (Cl) was significantly increased by PLA-NCs ($P < 0.05$), one could conclude that the colloidal carrier affected the rate of indomethacin elimination. This conclusion is supported by findings reported from an enterohepatic circulation study of indomethacin loaded PLA-NCs (Fawaz et al., 1993). In this investigation it was found that biliary secretion of the unmodified indomethacin following i.v. infusion of PLA-NCs was increased 2-fold as com-

Table 1
Pharmacokinetic and bioavailability parameters (mean values ± S.D.) following intravenous infusion and rectal administration

Parameters	Intravenous infusion		Rectal administration				
	Nanocapsule suspension	Solution	Nanocapsule suspension A	Nanocapsule suspension B	Solution A	Solution B	Suppository
C_{max} (mg/l)	9.2 ± 2.2	14.2 ± 3.6	8.1 ± 4.6	11.0 ± 4.1	11.6 ± 4.1	20.7 ± 7.8	14.2 ± 5.5
T_{max} (h)	1.3 ± 0.2	2	0.54 ± 0.1	0.5 ± 0.1	0.5	0.54 ± 0.1	0.5
$t_{1/2}$ (h)	5.7 ± 1.2	2.9 ± 0.7	4.8 ± 1.1	4.55 ± 0.98	4.25 ± 1.25	3.8 ± 1.9	6.1 ± 0.5
K_e (h^{-1})	0.127 ± 0.039	0.260 ± 0.085	0.219 ± 0.087	0.157 ± 0.032	0.175 ± 0.050	0.229 ± 0.112	0.171 ± 0.096
AUC_{0-24} (mg·h/l)	33.3 ± 5.7	46.3 ± 11.07	26.4 ± 7.5	35.7 ± 11.3	24.8 ± 10.5	31.7 ± 9.3	32.8 ± 10.7
$AUC_{0-\infty}$ (mg·h/l)	35.7 ± 6.4	46.52 ± 11.18	28.3 ± 8.3	36.7 ± 11.1	25.4 ± 10.7	32.2 ± 9.1	34.6 ± 10.6
Absolute bioavailability (%)	100	100	79[a]	103[a]	55[b]	69[b]	75[b]
Cl (ml/h)	288 ± 52	227 ± 60					
V_d (ml/kg)	2360 ± 580	920 ± 253					

Infusion and administration were of a dose equivalent to 10 mg of indomethacin per kg of the seven formulations (three solutions, three nanocapsule suspensions and suppository).

C_{max}, maximum plasma concentration; t_{max}, time to reach C_{max}; $t_{1/2}$, terminal half-life; k_e, constant of elimination rate; 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; Cl, total clearance; V_d, apparent volume of distribution.

[a]IV nanocapsule suspension was used as reference standard.

[b]IV solution was used as reference standard.

pared to solution. However, these results are not in agreement with those reported either by Maincent et al. (1986) from i.v. infusion of vincamine associated with polyhexylcyanoacrylate nanoparticles in rabbits, or more recently by Andrieu et al. (1989) after i.v. infusion of indomethacin loaded polyisobutylcyanoacrylate nanocapsules into rats. On the other hand, Ammoury et al. (1990) have found that in an artificial medium (pH 7.4), indomethacin was incompletely, but very quickly, released from PLA-NCs. Therefore, it may be expected that in blood, where release conditions are more suitable, the drug in PLA-NCs would be much more quickly released than in vitro. In this case, it is likely that PLA-NCs would have already lost part of their indomethacin load before being captured by the reticuloendothelial tissues. However, slow release of the drug from nanocapsules either in the blood or in the reticuloendothelial tissues cannot be disregarded since this would also explain the increased values of $t_{1/2}$ and V_d.

3.2.2. Rectal administration

Indomethacin plasma concentrations after rectal administration of solutions A and B and PLA-NC suspension A had similar profiles. A rebound in indomethacin plasma concentration, was observed 4–8 h after rectal administration of all dosage forms due, as for i.v. infusions, to enterohepatic circulation of indomethacin. However, this rebound was hardly noticeable with suppository perhaps because release and absorption of indomethacin in the rectum were slower and more progressive from suppository than from liquid formulations. Furthermore, solutions A and B, suspension A and suppository seemed to be bioequivalent and there was no significant difference between their $AUC_{0-\infty}$ values. However, the $t_{1/2}$ value for suppository (6.1 $\pm$ 0.5 h) was significantly longer than with other indomethacin formulations and absorption was more progressive with nanocapsules and suppository than with solutions. Thus no significant difference was found between the absorption of indomethacin from the solution and its absorption from PLA-NC suspension A. Since the pH of rectal liquid (7.2–7.4) is suitable for the release of indomethacin from nanocapsules, we suppose that some of the indomethacin would have been absorbed after it was released in free form in the rectum.

When indomethacin i.v. solution is used as the reference standard, the mean absolute bioavailability of indomethacin following rectal administration of solutions A and B was of 55 and 69%, respectively. These values indicated that absorption in both cases was not complete. However, the mean values of C_{max} following the administration of solutions A and B (11.6 $\pm$ 6.1 mg/ml and 20.7 $\pm$ 7.8 mg/ml, respectively) and the mean value of T_{max} (0.5 $\pm$ 0.1 h) indicated that the rate of absorption was fast. Furthermore, a significant difference was found between C_{max} values with solution A and solution B which could be attributed to the larger volume of solution B administered as compared to solution A. Therefore, a wider surface of rectal mucosa could have been covered by solution B with a probable overflow into the colon. On the other hand, a significant difference was found between the mean values of C_{max} after administration of solution B (20.7 $\pm$ 7.8 mg/ml) and the mean values of C_{max} with both nanocapsule suspensions A and B (8.1 $\pm$ 4.6 mg/l and 11.0 $\pm$ 4.1 mg/ml, respectively). However, there was no significant difference between C_{max} values obtained after administration of nanocapsule suspensions A and B.

No significant difference was found between mean values of the terminal half-life of indomethacin given in solution and loaded nanocapsules. Furthermore, the absolute bioavailability (F) of indomethacin after rectal administration of PLA-NC suspension B, was slightly higher than 100% (i.e. 103%). This result appears illogical because F was calculated with reference to the i.v. nanocapsule suspension. However, it must noted that there was no significant difference between the AUC_{0-24} values obtained after intravenous infusion and rectal administration of all nanocapsule suspensions. A similar increase in the absolute bioavailability following oral administration of indomethacin loaded polyisobutylcyanoacrylate nanocapsules to rats had already been reported by Andrieu et al. (1989) and attributed to variability in animal

groups. However, it is also likely that such a result would be due to an acceleration in the hepatic elimination rate of indomethacin after i.v. infusion of nanocapsules (uptake of nanocapsules by the reticuloendothelial system), whereas indomethacin given in nanocapsules by the rectal route was absorbed as it was released at the rectal site. Therefore, when the i.v. solution is used as reference standard, the absolute bioavailability of indomethacin in suspension A is only of 79%.

3.3. Protective effect study

Following intravenous infusion of indomethacin solution and PLA-NC suspension, no lesions of any kind were observed on the rectal mucosa of the rabbits. Therefore, there were no systemic side effects. Using the rectal route, whatever the indomethacin formulation, the administration of a single dose to rabbits was likely to be insufficient to induce ulcerative effects. On the other hand, a multiple dosage regimen was not considered due to the difficulty of fasting the rabbits for a longer period of time. Therefore, after rectal administration, no ulcerative effects were observed in all cases. Evidence of irritation was detected only on the rectal mucosa of rabbits to whom indomethacin or benzylbenzoate were administered in solution or in emulsion. The most important effect was found after rectal administration of indomethacin solution. In all animals, irritation was associated with many small rectal bleeding sites. The rectal wall of these rabbits had become thin as compared to control group to which normal saline solution was given. A similar irritation, but without bleeding, was also observed after rectal administration of the aqueous emulsion of benzylbenzoate.

Following the rectal administration of indomethacin free PLA-NCs only a slight and superficial irritation was detected in five rabbits. This reaction could be due to the release of free benzylbenzoate in the rectum. A very similar effect was also observed in all rabbits to whom a PLA-NC indomethacin suspension was given. A likely explanation for this is that indomethacin could have been absorbed quickly after its slow release in the rectum, thereby avoiding the formation of high indomethacin concentrations in the rectum and excessive local side effects. In this case, the free form of benzylbenzoate, released by the erosion of the nanocapsule wall, would be responsible for the irritation observed with PLA-NCs. However, there is no proof that rectal absorption of indomethacin was more rapid than absorption of benzylbenzoate.

Despite the limited results reported in this part of the study, the protective effect of PLA-NCs on the rectal mucosa against the local toxic effects of indomethacin and/or benzylbenzoate was evident. This finding is in agreement with the protective effect on the gastro-intestinal mucosa of some colloidal systems reported by many authors (Ammoury et al., 1989, 1991; Andrieu et al., 1986; Soehngen et al., 1988) and attributed to the prevention of direct contact of free drug with mucosa by nanoencapsulation. The absence of lesions on the rectal mucosa of rabbits to which indomethacin formulations were given by intravenous infusion suggested that protective effect of PLA-NCs is the result of a local process such as the slow release of the drug from PLA-NCs becoming a rate-limiting step for absorption. The small absorption area of the rectum as compared to the gastro-intestinal tract suggested that local concentrations of indomethacin would be more important in the rectum and thus the protective effect on rectal mucosa would not be as strong as for the gastro-intestinal mucosa.

4. Conclusions

In conclusion, the nanoencapsulation of indomethacin in PLA-NCs may allow more complete and progressive absorption of the drug by the rectal route and overcome some of the drawbacks of indomethacin at high local concentrations. A limited but interesting protective effect on the rectal mucosa against the irritating effect of indomethacin was shown. Protection would likely be better if benzylbenzoate was replaced by an other oily vehicle having lesser local toxic effects.

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