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Effect on Cerebral Blood Flow of Orally Administered Indomethacin-loaded Poly(isobutylcyanoacrylate) and Poly(DL-lactide) Nanocapsules

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Abstract—Nanocapsules, containing indomethacin, were prepared either by interfacial polymerization of isobutylcyanoacrylate monomers or by interfacial deposition of a preformed (DL-lactide) polymer. In-vitro release of indomethacin from nanocapsules was dependent on the pH of the sink solution and was enhanced by addition of albumin. A decrease in cerebral blood flow was noted 15 min after oral administration to rats of indomethacin nanocapsules (5 mg kg^{-1}) and lasted over 3 h. Empty nanocapsules had no effect. Since release of indomethacin from nanocapsules is unlikely to occur in the lumen of the stomach, due to unsuitable pH conditions, and nanocapsules have been previously shown to be able to cross the intestinal barrier, to reach the villi vessels intact and to protect against the ulcerating effect of the free drug, it is suggested that the rapid onset of the pharmacological effect was sufficiently induced by free indomethacin released in the plasma following absorption of the intact nanocapsules.

Colloidal drug-delivery systems, such as liposomes and nanoparticles, have been extensively studied, particularly in cancer chemotherapy. Much of this work has focused on drug targeting after systemic administration (Couvreur et al 1986). Less attention has been given to oral administration, due to poor stability of liposomes in the gastrointestinal tract (Woodley 1986) or poor absorption of polymeric nanoparticles (Nefzger et al 1984).

Recently, nanocapsules formed from a lipophilic droplet, as the core, surrounded by a thin wall (Rollot et al 1986) of polymeric material prepared by anionic polymerization of alkylcyanoacrylate monomer (Al-Khouri et al 1986) have been proposed as vesicular colloidal polymeric drug carriers.

The present study was initiated to evaluate the potential of indomethacin-loaded nanocapsules to deliver active drug systemically after oral administration. In addition to poly(isobutylcyanoacrylate) nanocapsules, a new type of poly(DL-lactide) nanocapsule (Fessi et al 1989) was also tested. For this purpose, the effect of indomethacin on decreasing the cerebral blood flow was selected, because of its rapid onset and its relation with cyclo-oxygenase inhibition (Pickard 1981).

Materials and Methods

Materials

Indomethacin was obtained from Sigma (USA), isobutylcyanoacrylate monomer was from Ethnor (France) and poly(DL-lactide), m. wt. 120000, from Phusis (France). All other chemicals were reagent grade chemicals and purchased from Prolabo (Paris, France).

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Animals

Experiments were carried out on male Sprague-Dawley rats (Dupre, France), 280–320 g, with free access to tap water and food pellets.

Nanocapsule preparation

Poly(isobutylcyanoacrylate) nanocapsules (PIBCA-NCs), either loaded or without indomethacin, were prepared as described by Al-Khouri et al (1986). Empty or drug loaded poly(DL-lactide) nanocapsules (PLA-NCs) were prepared following the process of Fessi et al (1989). Laser-light scattering (Super-nanosizer, Coultronics, France) was used for size measurements. Drug payload and drug loss in the aqueous suspending medium were assayed according to a modified HPLC technique (Drouet et al 1981). Stability testing involving size measurements, drug payload and drug loss following three-month storage at room temperature (20°C) was conducted. Free and encapsulated drug was determined following separation of nanocapsules from aqueous medium by an ultrafiltration technique (Centrisart I, Sartorius, West Germany).

In-vitro release study

Both types of nanocapsules were assayed for in-vitro release of indomethacin, using the dialysis sac diffusion technique (Ammoury et al 1989a). Indomethacin solution was used as a reference. The nanocapsules or solution to be tested (5 mL) were placed in the dialysis sac immersed in phosphate buffer 0·1 M (45 mL, pH 7·4), with or without albumin (10 mg mL^{-1}). The entire system was kept at 37°C with continuous magnetic stirring. Samples were taken at various intervals and assayed for indomethacin by HPLC. The regenerated cellulose membrane used (Spectrapor 12000) does not adsorb indomethacin.

Cerebral blood flow measurements

Cerebral blood flow (CBF) was measured using the hydrogen clearance method as modified by Haining et al (1968). Two weeks before the experiment, rats were chronically implanted within the frontal cortex, under chloral anaesthesia (300 mg kg^{-1} i.p.), with a glass-insulated platinized platinum electrode. The reference electrode was a silver wire implanted in the skull. Initial frontal CBF was measured 90, 60 and 30 min before treatment and CBF was measured 15, 30, 60, 90, 120, 150 and 180 min after treatment. All experiments were performed in conscious free-moving animals.

Treatments

Three groups of five rats each received indomethacin (5 mg kg^{-1} orally) either in 0·9% NaCl (saline) (treatment A), PIBCA-NCs (treatment B) or PLA-NCs (treatment C). The indomethacin concentration in the three dosage-forms was 1·25 mg mL^{-1} and rats received 4 mL kg^{-1}. Three groups (five rats each) were given an identical volume of either saline (treatment D) or an aqueous suspension of empty similar nanocapsules (treatments E and F, for PIBCA-NCs and PLA-NCs, respectively). The solution for treatment A was freshly prepared before use by dissolving indomethacin in 5 mg mL^{-1} sodium bicarbonate in distilled water.

Statistical analysis

A two-way analysis of variance (ANOVA) with and without repeated measurements, was used to test the stability and homogeneity of pre-dose CBF with time and between groups, and to study the time × group interaction.

A one-way ANOVA was used to test the homogeneity of the mean initial blood flow between groups.

Post-dose data were analysed by a two-way ANOVA with repeated measures to detect time × group interactions. The Newman-Keuls test was used to compare treatments at each post-dose time interval. A two-way ANOVA was also used to analyse pre-dose and post-dose CBF in each group followed by a Newman-Keuls test to compare each time-interval value.

Results

Nanocapsules

A monodisperse population of individual spherical nanocapsules was obtained by both preparation methods: 180 ± 24 nm for PLA-NCs and 220 ± 27 nm for PIBCA-NCs. High encapsulation efficiency was achieved and less than 3% of the initial indomethacin was found in the aqueous phase; the remaining 97% was incorporated in the colloidal carrier.

Both PLA and PIBCA nanocapsule suspensions remained stable for at least three months. No difference in size or drug content was observed and, owing to partition between the lipophilic core of the nanocapsules and the aqueous suspending medium (pH 3–4), no drug release occurred during storage. After three months, encapsulated indomethacin remained chemically unchanged, while an aqueous alkaline solution was significantly degraded during one week of storage at room temperature.

In-vitro release

The in-vitro release of indomethacin was negligible when pH in the receptor compartment was kept below 5. At physiological pH, 7·4, at which indomethacin becomes water-soluble, the release was slow and incomplete as a result of drug partition between the inner lipophilic phase of nanocapsules and the aqueous phase. When albumin was added to the receptor compartment, the release was faster and total, in accordance with the binding of the drug to albumin and the related increase of the concentration gradient of free diffusible species (Fig. 1).

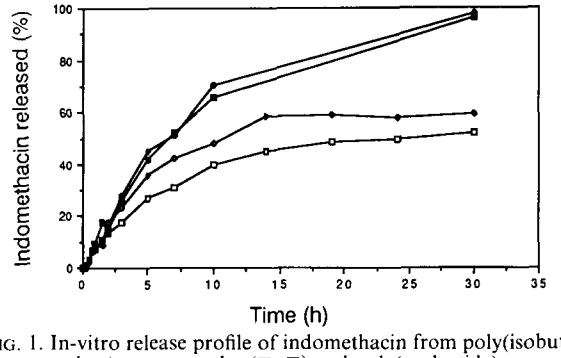

FIG. 1. In-vitro release profile of indomethacin from poly(isobutylcyanoacrylate) nanocapsules (□, ■) and poly(DL-lactide) nanocapsules (◇, ◆) at pH 7·4 (□, ◇) and in the presence of 10 gL^{-1} of albumin (■, ◆).

Cerebral blood flow (CBF)

CBF before treatment was stable and homogenous within time and groups, and no time × group interaction was detected (Table 1).

Post-treatment CBF did not differ from pre-treatment values in the control groups receiving treatments D, E or F (Fig. 2). In groups A, B and C, receiving indomethacin, a significant reduction in CBF was observed at each post-dose time interval (Fig. 2), although generally there was no significant difference amongst post-dose values.

Table 1. Cerebral blood flow values (CBF) as a function of time before treatment in the different rat groups. Each group comprised 5 rats; values are mean ± s.d.; Treatments A–F are as described in Materials and Methods.

Time (min)	Cerebral blood flow in rat groups, mL min^{-1}/100 g					
	A	B	C	D	E	F
90	92·6 ± 17·5	100·5 ± 20·7	118·9 ± 44·1	106·7 ± 38·3	109·8 ± 34·4	88·7 ± 10·7
60	81·5 ± 7·7	106·2 ± 28·8	116·9 ± 42·9	96·0 ± 35·2	105·4 ± 27·9	81·9 ± 6·4
30	85·9 ± 17·8	101·7 ± 12·4	105·8 ± 42·0	97·3 ± 34·2	84·9 ± 8·8	79·5 ± 8·3

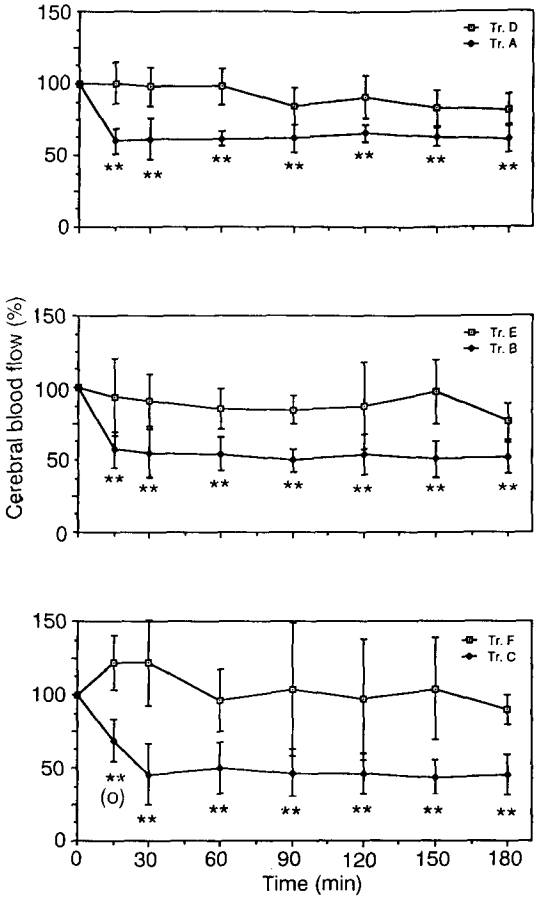

FIG. 2. Effect of various indomethacin preparations (5 mg kg $^{-1}$) and their respective controls on cerebral blood flow in conscious free-moving rats following oral administration: indomethacin aqueous solution (A) and saline (D); indomethacin-loaded (B) and empty (E) poly(isobutylcyanoacrylate) nanocapsules; indomethacin-loaded (C) and empty (F) poly(DL-lactide) nanocapsules. ** $P < 0.01$ versus pretreatment values; (o) $P < 0.05$ versus other post-treatment values.

Discussion

The results reported herein with respect to CBF reduction by indomethacin confirm the previous data in conscious free-moving rats (El-Bouchi et al 1985). No reduction of CBF was observed after oral administration of empty colloidal carriers, but both PLA and PIBCA indomethacin-loaded nanocapsules exhibited CBF reduction profiles similar to that observed when the drug was given as a solution. This may be attributed to the rapid and sensitive response of this pharmacological assay to indomethacin (Pickard & Mackenzie 1973; Dahlgren et al 1981). Thus, although the in-vitro release rate of the colloidal carriers was slower than that of free drug, it was still fast enough to achieve a threshold concentration at the first time point (15 min) in the in-vivo model. Furthermore, release in the circulation would be accelerated due to the presence of albumin and other components capable of acting as acceptors.

The rapid onset of this effect after oral administration of nanocapsules is in contrast to the 48 h lag-time noted after oral administration of insulin-loaded PIBCA-NCs (Damgé et al 1988). As insulin and indomethacin PIBCA-NCs were prepared by the same method and were in the same size range, different diffusion rates through the polymeric wall of nanocapsules, according to the different molecular weights and physicochemical properties of the drugs, cannot fully explain the difference in lag-times.

Lenaerts et al (1984) have shown that PIBCA nanoparticles were rapidly hydrolysed in-vivo to water-soluble compounds and it can be assumed that the thin polymeric wall, about 10 nm (Rollot et al 1986), of nanocapsules would be more rapidly degraded than the dense nanoparticle matrix. Therefore, a rapid release of the drug would be expected from nanocapsules in-vivo, either by diffusion (indomethacin) through, or biodegradation (insulin and indomethacin) of the polymeric wall.

Pharmacokinetic studies in rats showed similar plasma concentration profiles after intragastric infusion of indomethacin-loaded PIBCA-NCs and the free drug, with a more rapid absorption of the encapsulated drug in the first 4 h (Andrieu et al 1989). However, the assay method was not able to distinguish free from encapsulated drug in the plasma samples. It should be emphasized that the indomethacin solution would precipitate at the acidic pH of the stomach and under these conditions, the gastrointestinal absorption of free indomethacin as macroscopic crystals would be slower than that of a colloidal carrier.

In-vitro results showed that PLA- and PIBCA-NCs rapidly released indomethacin when physicochemical conditions were close to physiological conditions (pH 7·4 in the presence of albumin), while the release was poor at a pH below 5, conditions encountered in the stomach and proximal intestinal tract. Tolerance studies in pylorus-ligated rats have shown that nanoencapsulation protected the gastric mucosa from the ulcerative effect of indomethacin (Ammoury et al 1989b). This protective effect was also obtained throughout the intestine, including duodenum, jejunum and ileum, after repeated administration (Ammoury et al unpublished data), and similar results were observed with indomethacin-loaded liposomes given orally (Soehngen et al 1988). These results suggest that the local protection achieved with colloidal carriers such as nanocapsules or liposomes, despite the presence of systemic feedback of the drug, may be attributed to the reduction or lack of direct contact between the free drug and the surface of the mucosa.

Electron microscopy has shown the presence of intact PIBCA-NCs in intercellular spaces and defects of the mucosa, and in the lamina propria and the capillary bed of the villi, after intrajejunal administration to dogs (Aprahamian et al 1987). The results obtained with insulin-loaded PIBCA-NCs also confirm that nanoencapsulation can protect the drug from gastrointestinal degradation and make it available for a hypoglycaemic effect after oral administration (Dagmé et al 1988), suggesting that the carrier is neither degraded nor releases its content in the lumen. As yet, we do not have morphological evidence for the transfer of PLA-NCs. Recently, Jani et al (1989) have demonstrated that polystyrene micro- and nanoparticles cross the intestinal mucosa, so this phenomena may be common to many types of particle. PIBCA- and PLA-NCs do not have identical surface properties, but since both types of nanocapsules were prepared using the same surfactants, and since surfactant adsorption is known to modify the surface properties of particles, their behaviour in-vivo may be similar.

We have not yet obtained firm evidence that intact indomethacin nanocapsules cross the gastrointestinal mucosa; however, indomethacin nanocapsules protected the gastrointestinal mucosa from the irritating effect induced by free indomethacin without affecting the rate and intensity of the pharmacological activity. Such a protective effect could only be explained if free drug were not present in the gastrointestinal lumen in substantial quantities. Based on these previous observations, it is reasonable to suggest that the transport of indomethacin from the lumen to the blood could occur as a result of the passage of intact nanocapsules.

The only difference between PIBCA-NCs and PLA-NCs in the time course of CBF reduction was observed at 15 min. As the in-vitro release profiles of the two nanocapsule types are almost identical, some difference must exist in-vivo to explain the slower release of indomethacin from PLA-NCs. As previously mentioned, PIBCA is rapidly and enzymatically hydrolysed in-vivo to water-soluble compounds, suggesting that some drug could be initially released by diffusion but, soon after, the degradation of the polymeric wall should facilitate and accelerate its release. In contrast, PLA is known to undergo a slow non-enzymatic chemical hydrolysis (Salthouse et al 1976), and most of the drug would be released only by diffusion, at least the amount necessary to reach the threshold for the pharmacological response.

In conclusion, these results demonstrate the ability of orally administered polymeric nanocapsules, prepared either by a polymerization or a deposition process, to release indomethacin in a pharmacologically active form.

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