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The Effect of Site of Administration in the Gastrointestinal Tract on the Absorption of Insulin from Nanocapsules in Diabetic Rats

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Abstract—Isobutylcyanoacrylate nanocapsules have been used as drug carriers for the enteral absorption of insulin. Their absorption has been studied by measuring fasted glycaemia in streptozotocin-induced diabetic rats after a single administration of encapsulated insulin (100 units kg^{-1}) at various sites along the gastrointestinal tract. Glycaemia decreased from the second day, the intensity and duration depending on the site of administration (65% ileum, 59% stomach, 52% duodenum and jejunum, 34% colon). This hypoglycaemic effect lasted up to the 18th day after administration for ileum and jejunum, the 15th day for stomach and duodenum, and the 13th day for colon. In-vitro, nanocapsules protect insulin against proteolysis from pepsin, chymotrypsin and trypsin. These results suggest (i) that insulin is protected by nanocapsules in the gastrointestinal tract, (ii) that it is absorbed in an active form, and (iii) that ileum is the most potent site of absorption.

Among the many routes proposed for insulin administration, the oral route seems to be the most convenient if two conditions can be met (i) insulin must be protected in the gut from proteolytic degradation and (ii) insulin, a peptide composed of 51 amino acids, must be transported through the intestinal mucosa. To overcome these problems Saffran et al (1986) associated insulin to an azopolymer which is degraded by bacteria in the colon, thus releasing the insulin, while other workers have used liposomes (Dapergolas & Gregoriadis 1976; Patel & Ryman 1977; Weingarten et al 1981; Kimura et al 1984). We have chosen to associate insulin with another kind of colloidal drug delivery system, polyalkylcyanoacrylate nanocapsules (Damgé et al 1988). These are spherical structures less than 300 nm in diameter, surrounded by a biodegradable polymeric wall composed of isobutylcyanoacrylate (Al Khouri et al 1986). Previously we have found that nanocapsules less than 200 nm in diameter passed through the intestinal mucosa probably by a paracellular pathway (Aprahamian et al 1987). We have also discovered that insulin entrapped in nanocapsules remains biologically active after oral administration since it is able to decrease glycaemia in diabetic rats (Damgé et al 1988). Moreover, the duration but not the intensity of this biological response depends on the amount of nanocapsules administered, lasting up to 6 or 20 days with a single oral administration of 12·5 or 50 units kg^{-1} encapsulated insulin, respectively (Damgé et al 1988). To elucidate the mode of action of insulin associated to nanocapsules, we have studied in-vitro their protective properties against proteolysis and studied their intestinal absorption by measuring their biological effect as a function of time after delivery at various sites along the digestive tract.

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Materials and Methods

Preparation and characterization of nanocapsules

Insulin-loaded nanocapsules were prepared as previously described (Damgé et al 1988). In brief, 125 units of insulin (Velosulin, Nordisk, Denmark), and 125 mg of isobutyl-2-cyanoacrylate (Ethnor, Paris) were added to a lipophilic phase composed of 1 mL miglyol, a triglyceride formed from medium-chain fatty acids (Dyna-France, Paris) dispersed in 25 mL of pure ethanol. This phase was added with stirring to 50 mL of an aqueous solution containing 0·25% non-ionic surfactant (Poloxamer 188, ICI, Clamart, France). An emulsion of oil-in-water was formed and nanocapsules were obtained by polymerization of isobutylcyanoacrylate at the surface of the lipidic droplets. The suspension was then concentrated by evaporation under vacuum until reaching a concentration of 10 units insulin and 10 mg of polymer mL^{-1}. Purification of the suspension was achieved by filtration through fritted glass filters (9–15 μm). The calculated insulin/polymer ratio in the final suspension was 1 unit mg^{-1} of polymer.

For control experiments, an emulsion of insulin was prepared according to the same procedure, but in the absence of monomer.

The mean size and the intensity distribution pattern of nanocapsules were estimated using a laser light scattering method with a monochromatic laser ray diffusion counter (Autosizer 2C, Malvern Instruments, Les Ulis, France).

The encapsulation rate of insulin was calculated as the difference between the total amount of insulin and the amount of non-encapsulated insulin present in the suspension. The total amount of insulin in the preparation was determined after dissolution of nanocapsules with acetonitrile (v/v) for 24 h. The non-encapsulated insulin was measured in the polymerization medium after separation by ultracentrifugation at 35000 rev min^{-1} for 90 min. Insulin was determined in these two fractions using a reverse phase HPLC method.

Experimental procedures

In-vitro experiments. The stability of encapsulated insulin towards digestive enzymes was determined by evaluating the ratio of undegraded insulin after incubation of insulin-loaded nanocapsules in the presence of either pepsin or trypsin or α-chymotrypsin. The following enzymatic solutions were prepared; pepsin, 10 units mL^{-1} in 0·05 M glycine buffer, pH 1·4; trypsin, 7500 units mL^{-1} in 0·05 M phosphate buffer, pH 7·9; and α-chymotrypsin, 52 units mL^{-1} in 0·05 M phosphate buffer, pH 7·9.

A sample of nanocapsule suspension was mixed with an equal volume of each enzymatic solution in order to reach concentrations of 5 units insulin and 50 mg polymer per mL of incubation medium. Incubations were carried out at 37°C for 30 min. After incubation, the total amount of insulin was determined in the incubation medium after dissolution of nanocapsules with acetronitrile. Undegraded non-encapsulated insulin was determined after separation by ultracentrifugation at 35000 rev min^{-1} for 90 min. The amount of undegraded encapsulated insulin was calculated as the difference between the total amount of insulin in the incubation medium and the amount of undegraded non-encapsulated insulin originating from the nanocapsule suspension. Results were expressed as percentages of the initial amount of encapsulated insulin. Emulsion of insulin was used as a control in the same experimental conditions, results being expressed as percentages of the initial amount of insulin.

In-vivo experiments. Diabetes was induced in 48 male Wistar rats, 308 ± 3 g, by an intravenous injection of 65 mg kg^{-1} streptozotocin in citrate buffer, pH 4·5. Rats were considered as diabetics when fasted glycaemia was higher than 300 mg dL^{-1} one week after treatment.

Diabetic rats were divided into six groups of 8 animals each. Insulin-loaded nanocapsules or insulin emulsion were administered in a single dose after an overnight fast, at the rate of 100 units/100 mg polymer kg^{-1} for nanocapsules and 100 units kg^{-1} for emulsion. Group 1 received nanocapsules administered intragastrically by force feeding. Group 2 received nanocapsules injected in the duodenal lumen, below the pylorus. Group 3 received nanocapsules injected in the jejunal lumen below the ligament of Treitz. Group 4 received nanocapsules injected in the ileal lumen, 50 cm above the ileo-caecal valve. Group 5 received nanocapsules injected in the right colon. Group 6, the control group, received an emulsion of insulin injected either in the duodenum (n = 2), jejunum (n = 2), ileum (n = 2) or colon (n = 2).

In group 1, administration was performed in conscious animals, but groups 2 to 6 were anaesthetized with ether, and the intestinal segment was localized after laparotomy. The nanocapsule suspension or the emulsion was then injected through a canulae with a low delivery in order to allow a suitable diffusion along the intestinal segment and avoid local stretching.

The effect of insulin was recorded by measuring glycaemia for 20 days. Blood samples were taken from the retro-orbital sinus in overnight fasted animals before treatment, and at 2, 4, 6, 9, 13, 15, 18 and 20 days. Glycaemia was determined on plasma using the glucose oxidase method (Huggett & Nixon 1957).

Statistical analysis

In each group, glycaemia was expressed as means ± s.e.m. of 8 animals. Comparisons between animals treated with encapsulated and non-encapsulated insulin were performed using a one-way analysis of variance followed by a Newman-Keuls test.

Results

Characterization of insulin-loaded nanocapsules

The nanocapsule suspension appeared as a milky colloidal suspension. Estimated by laser light scattering, their size distribution appeared as homogeneous with a monomodal profile. The mean size of nanocapsules was 297 nm. The insulin emulsion was composed of 450 nm droplets also distributed homogeneously (Fig. 1).

The encapsulation rate of insulin, evaluated as the difference between the total amount of insulin in the suspension and the amount of non-encapsulated insulin, was 98%; the concentration of total insulin in the suspension was 10 units mL^{-1}.

Biological effect of insulin-loaded nanocapsules in diabetic rats

A single intragastric administration of insulin-loaded nanocapsules (100 units kg^{-1}) decreased glycaemia, measured after an overnight fast, by 59% ($P < 0·001$) from day 2 until day 9, after which glycaemia increased and reached control values after day 18 (Fig. 2).

After single intra-duodenal, -jejunal, -ileal and -colic injections of insulin-loaded nanocapsules (100 units kg^{-1}) (Fig. 3), similar profiles in plasma glucose concentrations were observed. However, the duration and intensity of the hypoglycaemic effect varied in relation to the site of administration. Thus the more obvious reduction in glycaemia was 51% of control values for duodenum, 53% for jejunum and 65% for ileum ($P < 0·001$) at day 4 following intra-luminal administration of 100 units kg^{-1} encapsulated insulin. However, this maximal reduction was only 34% of control values ($P < 0·01$) at day 2 after intra-colic administration. The hypoglycaemic effect observed was prolonged until day 15 after intra-duodenal injection, day 18 after intra-jejunal and -ileal injections, and day 13 after intra-colic injections.

In contrast, non-encapsulated insulin had no effect on glycaemia whichever site of administration was chosen.

The biological effect of insulin-loaded nanocapsules, in

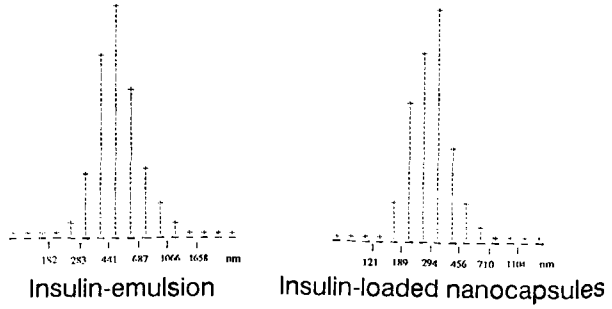

FIG. 1. Size distribution of insulin nanocapsules and insulin emulsion: profile of intensity distribution.

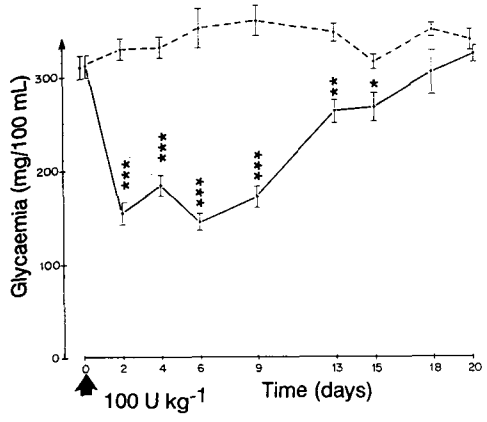

FIG. 2. Effect of a single intragastric administration of 100 units kg^{-1} insulin nanocapsules (—— n = 8) on glycaemia in diabetic rats. Results are means ± s.e.m. Comparisons calculated at each period against controls (- - - - n = 8), $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

decreasing plasma glucose level, was greatest in intensity and duration after intra-ileal administration, and lowest after intra-colic administration.

Protective properties of nanocapsules towards enzymatic degradation of insulin in-vitro

Non-encapsulated insulin incubated at 37°C for 30 min in presence of pepsin, chymotrypsin or trypsin, was found to be largely degraded. Only approximately 10% of the initial amount remained undegraded after incubation with pepsin and trypsin, and 35% after incubation with chymotrypsin.

By contrast, approximately 75% of the initial amount of encapsulated insulin was recovered after incubation with the three enzymes. Non-encapsulated insulin present in the nanocapsule was largely degraded.

Discussion

The present findings clearly indicate that insulin associated to a colloidal drug carrier, polyalkylcyanoacrylate nanocapsules, exerts a biological effect when administered enterally in diabetic rats. This hypoglycaemic effect lasted up to 11 or 16 days depending on the site of administration, along the digestive tract, for the single dose of 100 units of insulin per 100 mg of polymer (kg body weight)$^{-1}$. These results are consistent with our previous findings (Damgé et al 1988). Using an insulin nanocapsules suspension displaying an insulin/polymer ratio of 0·25 units (mg of polymer)$^{-1}$ fasted glycaemia was normalized from day 2 up to day 6, 9 and 20 with doses of 12·5 units/50 mg of polymer kg^{-1}, 25 units/100 mg of polymer kg^{-1} and 50 units/200 mg of polymer kg^{-1}, respectively. In the present work, we have prepared insulin nanocapsules with a higher insulin/polymer ratio (1 unit(mg of polymer)$^{-1}$) by increasing the amount of insulin during the polymerization process. The effect of an intragastric dose of 100 units/100 mg of polymer kg^{-1} tends to reproduce the previously observed effect induced by a dose of 25 units/100 mg polymer kg^{-1}, i.e. a normalization of fasted glycaemia from the 2nd up to the 9th day followed by a progressive recurrence of initial values. These data seem to confirm that

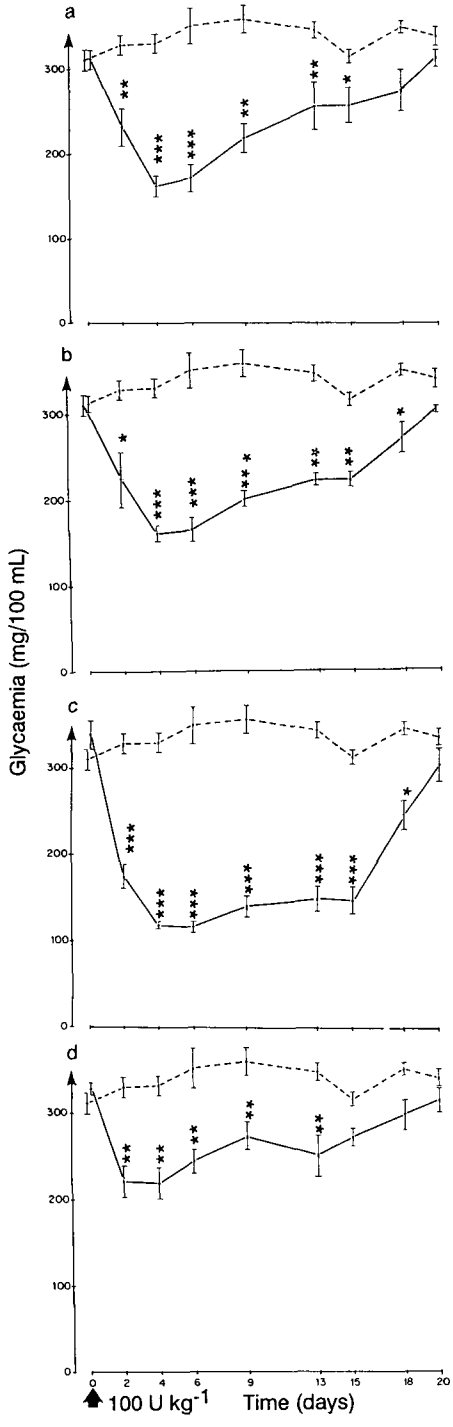

FIG. 3. Effect of a single intra-duodenal (a), -jejunal (b), -ileal (c) and -colonic (d) administration of 100 units kg^{-1} insulin nanocapsules (—— n = 8) on glycaemia in diabetic rats. Results are means ± s.e.m. Comparisons calculated at each period against controls (- - - - n = 8), $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

the biological effect of insulin nanocapsules depends on the amount of both insulin and polymer rather than on the amount of insulin alone.

The long-term hypoglycaemic effect of intragastric insulin nanocapsules may be explained in part by the progressive arrival of nanocapsules from the stomach to the gut, leading

to a delayed absorption. Indeed, after intubation of rats with radiolabelled polyalkylcyanoacrylate nanocapsules, Grislain et al (1983) found an intense labelling in the gastrointestinal tract after 4 h, the labelling still being present in the stomach after 24 h.

In the intestine, nanocapsules must play two roles; i, protection of insulin against proteolytic degradation and ii, transport of insulin from the intestinal lumen into the blood stream.

This study shows clearly that nanocapsules protect insulin against degradation by pepsin, trypsin and chymotrypsin in-vitro while non-encapsulated insulin was largely degraded in the same conditions. This implies that nanocapsules can play a protective role for insulin in the gut.

In order to discover the precise mechanism by which insulin nanocapsules are absorbed, we have administered them at various sites along the intestinal tract in diabetic rats and analysed their biological action. Our results suggest that all parts of the intestine are able to absorb insulin nanocapsules, with the insulin remaining active. The only variation observed was the intensity and duration of the hypoglycaemic effect. Finally, the rank of potency of the various absorption sites was ileum $\geqslant$ jejunum > duodenum > colon. This is surprising if it is assumed that the absorption of insulin nanocapsules is a function of the absorptive surface. Indeed, this is less important in the duodenum than in the jejunum, the segment being shorter. In fact, insulin nanocapsules administered to the upper part of the duodenum flow into the upper jejunum so that at least a part of their effect comes from jejunal absorption. Thus profiles of glycaemia were similar after intra-duodenal and intra-jejunal administration.

Our data also indicate that the ileum is more effective than the jejunum for insulin nanocapsule absorption. In both these segments, as well as in the duodenum, insulin linked to the drug carrier is the only way it can be absorbed and remain active. Indeed, when non-encapsulated insulin was administered in the stomach, duodenum, jejunum, ileum or colon there was no change in plasma glucose level. Thus insulin must be protected in the gastrointestinal tract against proteolytic enzymes as demonstrated by Kidron et al (1982). These authors found a 50% reduction in glycaemia when insulin combined with soybean trypsin inhibitor was administered in the ileum, while insulin alone was non-effective. In our study, nanocapsules formed by polymerization of isobutyl-2-cyanoacrylate in presence of insulin, are able to protect insulin. When insulin was adsorbed at the surface of polyalkylcyanoacrylate nanoparticles, which are full polymeric structures less than 300 nm in diameter, glycaemia was not reduced after oral administration in diabetic rats (Couvreur et al 1980). In these conditions, insulin can be easily desorbed from the polymeric drug carrier and destroyed in the gastrointestinal tract.

The main advantage of nanocapsules is that they are also able to transport the peptide from the gut lumen into the blood stream. As we reported previously (Aprahamian et al 1987), nanocapsules cross over the jejunal mucosa of the dog by a paracellular pathway probably using intercellular spaces formed by the desquamation of well differentiated absorptive cells at the tip of the villi. This passage is rapid, nanocapsules being recovered in the villus capillaries 30 min after intraluminal injection. A similar phenomenon could also occur in the ileum, but in this segment, a massive and rapid passage of nanocapsules through Peyer's patches, structures known for their role in the uptake of particulate matter, also occurred (Bockman & Cooper 1973), since 10 min after administration nanocapsules were found in the underlying lymph ducts (Defontaine et al 1989). Thus, although the total absorptive surface of the ileum is of less importance than that of the jejunum, the presence of numerous Peyer's patches could facilitate the absorption of insulin nanocapsules and account for the more obvious hypoglycaemic effect.

Finally, the poor ability of the colon to absorb insulin nanocapsules is understandable due to its poor absorptive surface. Insulin can pass through this epithelium but it must be associated to an adjuvant such as deoxycholic acid (Kidron et al 1982). In our experimental conditions, the presence of a surfactant, Poloxamer 188, in the nanocapsule suspension could facilitate the colic absorption of either free insulin or insulin released from nanocapsules. This hypothesis could explain the low hypoglycaemic effect of insulin nanocapsules administered in the rat colon.

After enteral absorption, insulin nanocapsules may be distributed in the organism, stored in the target organs and degraded progressively, releasing insulin. The most probable way of degradation of the polymer may be by enzymatic hydrolysis of the polymeric side ester chains leading to the solubilization of the polymer. As shown in-vitro, esterases are implicated in this degradation pathway (Lenaerts et al 1984).

In conclusion, these results indicate that polyalkylcyanoacrylate nanocapsules are able to protect insulin from proteolytic degradation in the gastrointestinal tract and transport it from the gut lumen into the blood stream, the insulin remaining biologically active for a certain time. Absorption of insulin associated to nanocapsules occurs in all parts of the gut to various degrees, but the most effective site of absorption seems to be the ileum.

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