ELSEVIER

International Journal of Pharmaceutics 242 (2002) 325–328

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Note

Absorption and efficiency of insulin after oral administration of insulin-loaded nanocapsules in diabetic rats

F. Cournarie [a], D. Auchere [b], D. Chevenne [c], B. Lacour [b], M. Seiller [a], C. Vauthier [a,*]

[a] *Laboratoire de Physico-Chimie, Pharmacotechnie et Biopharmacie UMR CNRS 8612, Université Paris XI, Faculté de Pharmacie, 5 rue Jean-Baptiste Clément, 92 296 Châtenay-Malabry Cedex, France*

[b] *Laboratoire de Physiologie, UPRES 2706, Université Paris XI, Faculté de Pharmacie, 5 rue Jean-Baptiste Clément, 92 296 Châtenay-Malabry Cedex, France*

[c] *Laboratoire de Biochimie Hormonale, Hôpital Robert Debré, Paris, France*

Received 26 December 2001; received in revised form 10 January 2002; accepted 1 February 2002

Abstract

Poly(isobutylcyanoacrylate) nanocapsules have been shown to decrease the blood glucose level after oral administration to streptozotocin-induced diabetic fasted rats after 2 days [Diabetes 37 (1988) 246]. Yet, the absorption of insulin in the blood of rats has not been characterised. The aim of this work was to evaluate the biological activity of insulin given orally as nanocapsules. Humalog®-loaded nanocapsules (50 IU/kg) were administered by gavage to streptozotocin-induced diabetic rats. Thirty minutes to 1 h after oral administration, significant levels of human insulin were detected in rat plasma. However, the concentrations were very heterogenous from one rat to another and no decrease of glycemia could be observed. In addition, parenteral injection of insulin in solution showed that high levels of the protein are necessary to decrease blood glucose concentration in diabetic rats. These concentrations were not reached after oral administration. The same dose of insulin decreased glycemia by 50% in normal rats and by only 25% in diabetics. This suggested that an insulino-resistance was developed by streptozotocin-induced diabetic rats.

Keywords: Insulin; Poly(isobutylcyanoacrylate) nanocapsules; Oral administration; Parenteral injection; Diabetic rats

Insulin is the most important regulatory hormone implicated in the control of glucose homeostasis. More than 30 million people around the world suffer from insulin-dependant diabetes mellitus and require daily parenteral injections of insulin. Pharmacokinetics of traditional insulin preparations don't reproduce the endogenous profile of insulin and it is almost impossible to maintain a normoglycemia (Zinman, 1989). As a

* Corresponding author. Tel.: +33-1-4683-5386; fax: +33-1-4661-9334

E-mail address: christine.vauthier@cep.u-psud.fr (C. Vauthier).

0378-5173/02/$ - see front matter
PII: S0378-5173(02)00175-8

consequence, insulin treated diabetics frequently manifest wide fluctuations in plasma blood glucose. Secreted by the pancreas into the portal vein, the protein reaches directly the liver which extracts a large fraction of it before its entry into the systemic circulation (Honey and Price, 1979). If peripherally administered insulin is not physiologic, the oral route will mimic the first hepatic uptake. However, several problems must be overcome before proteins could be efficiently administered orally such as proteolytic breakdown in the gastrointestinal tract and absorption through the intestinal epithelium. Drug carriers such as poly(-isobutylcyanoacrylate) nanocapsules have been shown to decrease the blood glucose level 2 days after oral administration to streptozotocin-induced diabetic fasted rats (Damgé et al., 1988). Indeed, the biological response induced by the oral administration of insulin-loaded nanocapsules was evaluated in terms of blood glucose levels and the absorption of insulin was not characterized.

The aim of the present study was to evaluate biological activity and bioavailability of insulin given orally as insulin-loaded nanocapsules.

Insulin-loaded nanocapsules were prepared by interfacial polymerization of isobutylcyanoacrylate (Al. Khouri Fallouh et al., 1986) adapted to the encapsulation of insulin by Damgé et al. (1988). This method consists of adding under magnetic stirring, an organic phase containing insulin, absolute ethanol, Miglyol 812® (medium-chain triglycerides) and isobutylcyanoacrylate (monomer) to an aqueous phase containing Lutrol® F68 as a surfactant. The suspensions of nanocapsules were adjusted to 10 IU insulin/10 mg polymer/ml by rotoevaporation under vacuum. The mean nanocapsules diameter, estimated by Quasi Elastic Light Scattering, was 400 ± 90 nm. The insulin used was Humalog®, a recombinant human insulin analog. Insulin encapsulation efficiency, determined by HPLC using a C18 reverse phase column, was 90%. Diabetes was induced by intraperitoneal injection of 65 mg/kg of streptozotocin in 35 male Wistar rats (300 g). One week after the induction of diabetes and 24 h before experiment, a catheter was implanted in the carotid and tunneled sub-cutaneous under the neck. With the intention of determining the insulin-loaded nanocapsules biological activity, three independent experiments (three groups of ten rats) were performed in which fasted diabetic rats received 50 IU/kg of insulin-loaded nanocapsules by gavage. Control diabetic rats received nanocapsules without insulin. Blood samples were collected during 5 hours the first day and once a day on subsequent days. Specific dosages of the human insulin were performed on the plasma using the BI-Insulin IRMA kit from SANOFI-Pasteur Diagnostics. Glycemia was determined using an Hitachi® 911 analyser.

The rats mortality was 30%, 1 week after the induction of diabetes, due to the hyperglycemia. As the dosage of insulinemia is specific for human insulin and does not cross react with residual rat insulin, measured insulin levels in rat plasma represent insulin absorbed from nanocapsules. The kinetic studies showed detectable plasma levels of human insulin in every treated rat for 5 h after the peroral administration of insulin-loaded nanocapsules. However, the measured concentrations of insulin were very variable from one animal to another. Two kinds of rats were distinguished according to the level of insulinemia. Rats showing human insulin level higher than 20 mIU/l were named 'high absorption type' whereas those in which human insulin was lower than 20 mIU/l were called 'low absorption type'. This arbitrary value was chosen because plasma insulin levels were found around 20 mIU/l in fasted normal rats (results not shown). It is then expected that above 20 mIU/l, insulin exerts its biological activity that is to decrease the glycemia. Individual results were represented for each rat in the 'high absorption type' whereas the mean was represented for those of the 'low absorption type' (Fig. 1). The peak of insulin absorption appeared 1 h after gavage in the first experiment (blood was not sampled at 30 min), 30 min for the second experiment, whereas no peak was observed in rats of the third experiment. Plasma insulin concentrations ranged from 50 ± 20 mIU/l in the first experiment to 240 mIU/l for one rat in the second experiment. Undetectable levels of human insulin were measured in controls. The days after, no more insulin was detected.

Those results show that (1) oral administration of nanocapsules allows to deliver noticeable levels of insulin into the blood of diabetic rats; (2) intestinal absorption of nanocapsules is heterogeneous; and (3) insulin is absorbed very quickly. This is in agreement with the fact that nanocapsules are transported along the small intestine in less than 1 h (Aboubakar et al., 2000). Furthermore, Humalog® is of the faster insulin absorption type. Predominantly hexameric in the preparation, this insulin dissociates faster than regular insulin into dimers and then into monomers, the only active form of insulin.

Surprisingly, there was no correlation in this model between insulinemia and glycemia. No change in blood glucose concentration appeared, neither during the peak of insulin, nor 2 days after administration. In term of biological activity, those results are not consistent with those of Damgé et al. (1988), who described a hypoglycemic effect after 2 days, assuming a delayed absorption of insulin. However, in the work of Damgé et al. (1988), plasma insulin levels were not measured. Nevertheless, in term of intestinal absorption, our results are in agreement with those of Lowe and Temple (1994) who detected insulin in the rat serum 30 min after intraduodenal administration of the same nanocapsules.

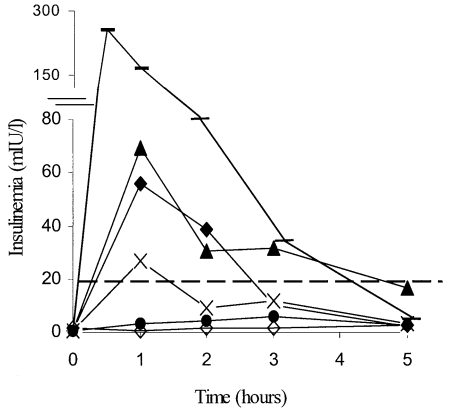

Fig. 1. Evolution of insulinemia after oral administration of 50 IU/kg of Humalog®-loaded nanocapsules, -——, rat 1 'high absorption type'; ▲, rat 2 'high absorption type'; ◆, rat 3 'high absorption type'; ×, rat 4 'high absorption type'; ●, 'low absorption type' ($n = 7$); ◇, controls ($n = 5$).

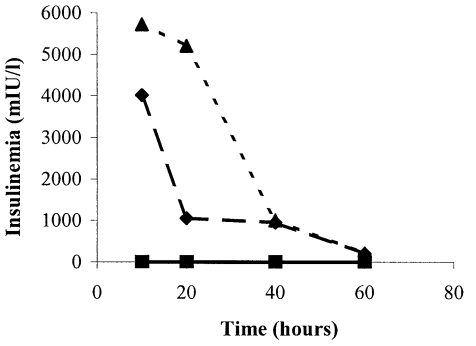

Fig. 2. Evolution of insulinemia after intra-arterial injection of 5 IU/kg of Humalog® in normal and diabetic rats, ◆, diabetic rats ($n = 2$); ▲, normal rats ($n = 2$); ■, controls ($n = 2$).

From such results, one can postulate that the encapsulated insulin was not biologically active. Thus, the hypoglycemic activity of insulin was evaluated after parenteral administration. Normal and diabetic feed rats were given 5 IU/kg of insulin in solution through the implanted catheter. The catheter was then washed with physiological serum to avoid any contamination during sampling. Humalog® decreased glycemia by 50% at 40 min in normal rats and by only 25% between 20 and 40 min in diabetic rats. Plasma human insulin increased up to 5500 mIU/l in normal rats whereas it only increased up to 4000 mIU/l in diabetics (Fig. 2).

Those results show that (1) high levels of plasma insulin were necessary to produce an efficient hypoglycemic activity of insulin and were not reached after oral administration of nanocapsules; (2) the hypoglycemic activity of insulin was lower in diabetic rats compared to normal ones; and (3) circulating rates of insulin were lower in diabetic rats than in normal ones, suggesting a higher consumption or a higher fixation on insulin receptors. The low biological effect of insulin observed in diabetic rats while the circulating levels of insulin were higher, suggests that an insulino-resistance appeared. Blondel and Portha (1989) described an early insulino-resistance that develops in hepatic tissues 24 h after streptozotocin injection and expands 9 days after to peripheral tissues. Actually, in our work, we performed

the experiments 1 week after the induction of diabetes.

This work has proved that insulin encapsulated in poly(isobutylcyanoacrylate) nanocapsules can cross the intestinal wall and be absorbed in the blood at significant concentration. The absence of biological activity, that could be the result of the development of an insulino-resistance, lead us to wonder if streptozotocin-induced diabetic rats constitutes a suitable model to study the oral efficiency of the insulin encapsulated in nanocapsules.

Acknowledgements

We are indebted to the Eli Lilly Company (Indianapolis, USA) for a generous supply of Humalog®. We are very grateful to Loctite Compagny (Dublin, Ireland) for the supply of Isobutylcyanoacrylate. F. Cournarie was a fellow from the CNRS and Assistance Publique des Hôpitaux de Paris.

References

Aboubakar, M., Couvreur, P., Pinto-Alphandary, H., Gouritin, B., Lacour, B.R.F., Puisieux, F., Vauthier, C., 2000. Insulin-loaded nanocapsules for oral administration: in vitro and in vivo investigation. Drug Dev. Res. 49, 109–117.

Al. Khouri Fallouh, N., Roblot Treupel, L., Fessi, H., Devissaguet, J.P., Puisieux, F., 1986. Development of a new process for manufacture of polyisobutylcyanoacrylate nanocapsules. Int. J. Pharm. 28, 125–132.

Blondel, O., Portha, B., 1989. Early appearance of in vivo insulin resistance in adult streptozotocin-injected rats. Diab. Metab. 15, 382–387.

Damgé, C., Michel, C., Aprahamian, M., Couvreur, P., 1988. New approach for oral administration of insulin with polyalkylcyanocrylate nanocapsules as drug carrier. Diabetes 37, 246–251.

Honey, R.N., Price, S., 1979. The determinants of insulin extraction in the isolated perfused rat liver. Horm. Metab. Res. 11, 111–117.

Lowe, P.H., Temple, C.S., 1994. Calcitonin and insulin in isobutylcyanoacrylate nanocapules: protection against proteases and effect on intestinal absorption in rats. J. Pharm. Pharmacol. 46, 547–552.

Zinman, B., 1989. The physiologic replacement of insulin: an elusive goal. N. Engl. J. Med. 321, 363–370.