J. Pharm. Pharmacol. 1980, 32: 108–110 Received December 28, 1978

Effective intestinal absorption of insulin in diabetic rats using a new formulation approach

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Insulin injected intra-jejunally together with the non-ionic surfactant cetomacrogol was effective in streptozocin-induced diabetes in the rat, as measured by the hypoglycaemic effect. The reduction in blood sugar was maximal at about 2 h after administration but continued at a high level for the 4 h of the experiment. No hypoglycaemic effect was observed in controls injected with insulin or saline alone. Intestinal absorption of insulin has thus been effected by the addition of cetomacrogol, which appears to enhance membrane-permeability to insulin rather than to function as a protective agent preventing insulin degradation, as in liposome-encapsulation. In support of this, a significant hypoglycaemic action was still obtained when the insulin injection was given half-hour after that of the cetomacrogol, both intra-jejunally. Furthermore, oral administration of the surfactant followed by intra-jejunal injection of the insulin also gave a hypoglycaemic effect. The use of this agent to enhance insulin absorption offers the possibility of a new approach to oral insulin therapy.

The therapeutic administration of peptides such as insulin is at present limited to the injection route since these agents appear to be destroyed in the gastrointestinal tract. Diabetic patient compliance with a self-injection regime is a problem which has led to much research, hitherto unsuccessful, on finding a method for oral administration of insulin.

Although certain intact protein molecules can be absorbed from the gastrointestinal tract, the extent of insulin absorption is extremely small (Crane & Luntz 1968). By use of up to 35 units of insulin in the presence of a trypsin inhibitor, Laskowski et al (1958) obtained some absorption from rat isolated intestinal loops. Other investigators also detected some insulin absorption using the everted intestinal sac technique with soya bean trypsin indicator, indole-3-acetate or diisopropylfluorophosphate (Danforth & Moore 1959). Patel & Ryman (1976), encapsulated insulin in a liposome, the phospholipid walls of which were thought to protect the contents. These studies exemplify attempts to increase insulin absorption by avoiding its destruction by peptidases.

Considering inactivation and absorption as simultaneous competitive processes, the low biological activity of insulin by the gastrointestinal route could be due to the unfavourable ratio of the rates of the two processes. If absorption rate could be increased to become dominant, the degree of inactivation during absorption would be relatively insignificant; this would present a new approach to insulin therapy.

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The aim of this study was to investigate the intestinal absorption of insulin in the presence of a non-ionic surfactant. A number of reports in the pharmaceutical literature show that surface-active agents can alter the magnitude of absorption of certain drugs (Gibaldi & Feldman 1970; Davis et al 1970). Intestinal, gastric or rectal absorption of insulin was increased in animals, and in some cases man, by several ionic surface-active agents; however, considerable local irritation occurred with these ionic agents and could not be relieved (Hanzlik & Cutting 1941). Non-ionic surfactants are widely used in pharmaceutical formulations as solubilizing agents and emulsifiers and are reported to have a low toxicity orally (Swisher 1968). In the work presented here cetomacrogol 1000 (n-hexadecyl polyethylene (20–24) ether), which is official in the B.P. Codex, was selected for study (Touitou et al 1978). The insulin was introduced intra-jejunally into diabetic rats, this region being chosen because of its lower protease content, relative to stomach and duodenum, and its higher pH, with the aim of reducing the degree of degradation. The extent of absorption was estimated from the reduction in blood glucose.

MATERIALS AND METHODS

Animals

Male 'Sabra' Hebrew University-strain rats (230–270 g) fed on pelleted chow diet became insulin deficient 72 h after injection of streptozocin (50 mg kg^{-1} weight) (Patel 1974). The intestine was exposed by a midline abdominal incision and intra-jejunal injections were made 5 cm postypylorically.

Treatment

Intra-jejunal injection of a solution containing insulin together with the non-ionic surfactant, was carried out as follows: The sample solution comprised 2 g of cetomacrogol 1000 (ABM, Cheshire, U.K.) with 10 ml of neutral insulin (80 u ml^{-1}) (Leo Neutral, Nordisk Insulin Laboratorium, Copenhagen, Denmark). Two controls were used, 0·9% NaCl (normal saline) solution and neutral insulin solution (80 u ml^{-1}). The quantities of the sample and control solutions administered by direct injection into the jejunum were 0·5 ml, which contained 35·1 i.u. insulin and 87·7 mg cetomacrogol 1000. Also 1 ml of a control of 4 u ml^{-1} of neutral insulin was given intraperitoneally to four diabetic rats. A solution, 0·5 ml, of 2 g cetomacrogol in 10 ml water was given under anaesthesia orally via a rubber tube and after 30 min, when the rats were not anaesthesized, 0·5 ml of neutral insulin solution (80 u ml^{-1}) was injected intrajejunally as before.

Blood was collected from the rat tails 1 h before and at 0, 1, 2 and 3 or 4 h after insulin administration. The rats were ether-anaesthesized intermittently during the stages of administration of drug and controls and during blood collection. Plasma glucose was determined at 610 nm using the GOD-Perid method (Werner et al 1970).

RESULTS AND DISCUSSION

Intra-jejunal administration of the solution containing insulin and cetomacrogol together to diabetic rats gave a large reduction in blood glucose concentration for at least 4 h (Table 1). The maximum hypoglycaemic effect occurred at 2 h after dosing, when the blood glucose content was 21·0% ± 2·0 (mean ± s.e.m.) of the initial value; after this the glucose concentration started to return towards normal values. This response is similar to that given by intraperitoneal injection of 4 i.u. (Fig. 1).

Solution of insulin alone given intra-jejunally caused no glucose reduction. This is as expected on the accepted basis that insulin undergoes rapid denaturation and proteolytic degradation, absorption being insignificant during decomposition.

The very high significance level $P < 0{\cdot}001$ of the results for the insulin-surfactant mixture, both against insulin alone and against the saline controls, shows that systemic absorption of the insulin is promoted by the presence of the surfactant.

With regard to the mechanism of the effect, there is the possibility that it might be due to protection of the insulin from degradation. The activity of proteolytic enzymes is very unlikely to be reduced by

Table 1. Effect of cetomacrogol on intrajejunal absorption of insulin in diabetic rats.

Sample administered†	Initial blood glucose conc mg % (mean ± s.e.m.)	Blood glucose at times after insulin administration‡ % of initial content (mean ± s.e.m.) 1 h	2 h	4 h
Saline (n = 4)	326 ± 17·0	97 ± 2·9	96 ± 5·0	90 ± 4·3
Insulin (n = 5)	296 ± 18·8	104 ± 3·0 $P^* < 0{\cdot}2$	100 ± 3·5 $P^* < 0{\cdot}6$	95 ± 2·0 $P^* < 0{\cdot}4$
Insulin-cetomacrogol (n = 9)	309 ± 14·3	56·5 ± 2·8 $P^{**} < 0{\cdot}001$	21·0 ± 2·0 $P^{**} < 0{\cdot}001$	37·7 ± 4·1 $P^{**} < 0{\cdot}001$

† Each run was carried out on a different animal; n = no. of rats. For sample composition see text.

‡ Blood glucose content (% of initial) after interperitoneal injection of 4 i.u. of insulin was 36·1 ± 2·2, 28·7 ± 1·0, 39·4 ± 3·6 at 1, 2 and 4 h respectively (4 rats). Initial blood glucose concentration in mg %: 315 ± 32 (mean ± s.e.m.).

* Insulin vs saline.

** Insulin-surfactant vs saline or insulin (same P values).

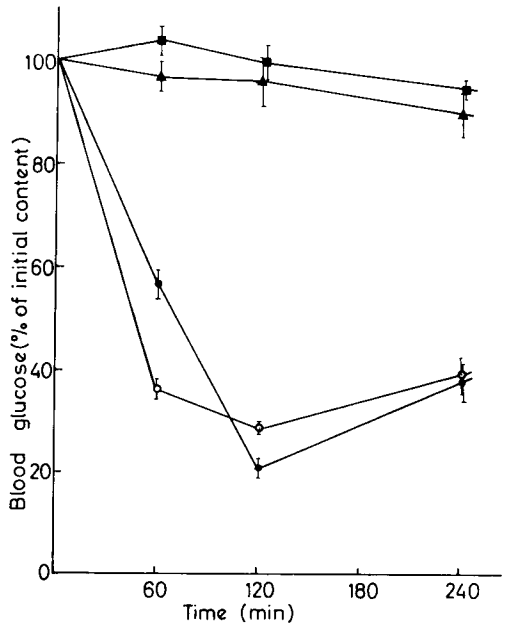

FIG. 1. Hypoglycaemic effect of insulin-cetomacrogol administered intrajejunally to diabetic rats: comparison with insulin and saline controls and intraperitoneal injection of insulin. (For dosage, see text). ● insulin-cetomacrogol i.j. ○ insulin i.p. ▲ saline i.j. ■ insulin i.j.

the non-ionic surfactant. Although surfactant micelles could isolate solubilized molecules or reduce their contact with enzymes, micellar systems would be entirely different structurally from liposome-entrapped insulin (Patel & Ryman 1976) in which droplets of an aqueous insulin solution or suspension are encapsulated in a lipophilic phospholipid wall material. Our micelles, which possess a lipophilic core

and hydrophilic polyetheric mantle containing free and hydration water (Elworthy et al 1968), do not exist as static microscopic bodies but are in dynamic equilibrium with monomer surfactant and form a clear aqueous solution at the concentration used. Shielding of labile peptide groups from enzymatic action would require protective incorporation within a molecular complex, micelle or similar aggregate, a notion which is not readily compatible with the chemical structure of insulin, a non-amphiphilic, non-lipophilic self-aggregated peptide. In fact, when the surfactant-insulin mixture was administered orally at the same dose as given intra-jejunally, there was no hypoglycaemic activity.

In any event, even if an insulin-surfactant micelle were formed, micellar solubilization has been shown to impair rather than aid the absorption of drugs, the high mol. wt drug-micelle combination being unable to penetrate the membrane to a significant extent (Gibaldi & Feldman 1970). Other experiments have shown that effective systemic absorption of insulin is promoted using cetomacrogol in semi-solid bases applied rectally or by the vaginal route, where, in the absence of peptidases, protection of the insulin molecule would not explain the promoting action (Touitou et al 1978). This raises the possibility that the surfactant influences membrane permeability.

To prevent direct contact of insulin and surfactant and provide some time for transportation of the surfactant away from the site before injection of the insulin at the same site, the surfactant and insulin solution were also injected separately with a time interval between. The surfactant solution, unlike the rectal base, has a high fluidity which is not appreciably different from that of water. The results obtained by intra-jejunal injection of cetomacrogol, followed after 30 min by intra-jejunal injection of insulin, showed a delayed but pronounced reduction in blood glucose concentration, reaching 58·4% ± 6·1 of the initial values at 3 h ($P < 0.01$) using the same dosage as in the insulin-surfactant mixture. When the surfactant was given by mouth by means of a flexible rubber tube and the insulin injected intra-jejunally after 30 min, the effect was less strong (74·0% ± 5·4 of initial blood glucose concentration, at 1 h, rising progressively to 83·2% ± 0·1 at 3 h). The reason for the variation in time of maximum activity is not self evident and may be connected with the fact that no attempt was made at this stage to control the factors influencing the rate of transportation of the respective solutions through the gastrointestinal system. Nevertheless, significant hypoglycaemic activity was observed on giving the insulin intra-jejunally 30 min after treatment with surfactant. The mechanism is evidently associated with change in the membrane permeability to insulin (c.f. Davis et al 1970; Birkett & Silen 1974).

The results of this work indicate that insulin absorption might be accomplished by oral administration of a suitably designed product containing insulin and surfactant, provided that the insulin were protected against degradation by a suitable coating during its passage to the jejunal absorption site.

The use of the non-ionic surfactant thus provides a new approach to the enteral absorption of insulin different from liposome-encapsulation but with retention of hypoglycaemic activity and excellent reproducibility under test conditions of application reported here. It opens a possible way towards the development of alternatives to prevailing insulin parenteral therapy.

Acknowledgments

The authors thank Mrs Dalia Gilhar for technical assistance in the blood glucose assays.

REFERENCES

Birkett, M. B., Silen, W. (1974) Gastroenterology 67: 1131–1138

Crane, C. W., Luntz, G. R. W. N. (1968) Diabetes 17: 625–627

Danforth, E. Jr., Moore, R. O. (1959) Endocrinology 65: 118–123

Davis, W. W., Pfeiffer, R. R., Quay, J. F. (1970) J. Pharm. Sci. 59: 960–963

Elworthy, P. H., Florence, A. T. MacFarlane, C. B. (1968) Solubilization by Surface-Active Agents, Chapman & Hall, London

Hanzlik, P. J., Cutting, W. C. (1941) Endocrinology 28: 368–374

Gibaldi, M., Feldman, S. (1970) J. Pharm. Sci. 59: 579–589

Laskowski, M. Jr., Haessler, H. A., Miech, R. P., Peanasky, R. J., Laskowski, M. (1958) Science 127: 1115–1116

Patel, H. M. (1974) Ph.D. Thesis, p. 69 University of London

Patel, H. N., Ryman, B. E. (1976) FEBS Letts 62: 60–63

Swisher, R. D. (1968) Arch. Environ. Health 17: 232–246

Touitou, E., Azaz, E., Bar-On, H., Donbrow, M., Eldor, A., Kidron, M., Lichtenberg, D., Ziv, E. Patent applied for.

Touitou, E., Donbrow, M., Azaz, E. (1978). J. Pharm. Pharmacol. 30: 662–663

Werner, W. H., Rey, G., Wielinger, H. (1970) Fresenius Z. Anal. Chem. 252: 224–228