ELSEVIER

International Journal of Pharmaceutics 119 (1995) 127–131

international journal of pharmaceutics

Research Papers

Development of experimental insulin pumps with glucose-controlled release

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Received 17 August 1994; accepted 21 October 1994

Abstract

In the present investigation, experimental insulin pumps have been developed in which the release of insulin is governed by the glucose concentration. The mechanical energy required for the glucose-dependent emptying of the insulin reservoir is provided by the carbon dioxide formed on the interaction of glucose with yeast cells. The developed pumps consist of a delivery unit containing an insulin reservoir and a reaction chamber in which 50 mg freeze-dried yeast and 150 μl glucose solution are incubated. Variable glucose-dependent insulin release could be obtained by using an exchangeable bioreactor, which is replaced at given intervals by a new bioreactor containing fresh supplies of freeze-dried yeast and glucose solution. The results with solutions of 100, 200, and 400 mg glucose per 100 ml show that insulin release kinetics are directly affected by the glucose concentration.

Keywords: Insulin delivery system; Glucose-controlled insulin release; Biologically controlled system; Yeast

1. Introduction

Drug delivery systems in which the release of drug substances can be actively controlled will become increasingly important in the future. In feedback-controlled closed loop dosage systems, release is governed by the individual requirements of the patients (Baker, 1987).

In earlier studies on the variable regulation of drug release, our research group has been concerned with the development of small sized drug delivery systems in which electrical circuits are used to control release (Gröning et al., 1991; Gröning, 1992). A complete computerized system for a feedback-controlled drug release, including monitoring and control devices requires a considerable outlay on apparatus (Gröning and Weyel, 1993). In order to miniaturise a feedback-controlled delivery system it is necessary to develop new principles of control.

In the last few years, several research groups have tried to develop small sized glucose-controlled insulin systems (Heller, 1993) using a variety of approaches. For example, glucose oxidase-containing systems (Horbett et al., 1983; Ishihara et al., 1984; Albin et al., 1985; Ishihara and Matsui, 1986; Albin et al., 1987; Ito et al., 1989; Chandy and Sharma, 1992) have been constructed. Another self-regulated insulin delivery

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SSDI 0378-5173(94)00376-9

system utilizes the glucose-glucose dehydrogenase reaction to release insulin (Chung et al., 1992). The competitive binding of glucose and glycosylated insulin to the lectin concanavalin A has also been used to control insulin release (Brownlee and Cerami, 1979, 1983; Sato et al., 1984; Kim et al., 1990; Makino et al., 1991; Pai et al., 1992; Kim and Jacobs, 1994). Other investigations have studied the affinity between boronic acid moieties and glucose (Choi et al., 1992; Kitano et al., 1992; Shiino et al., 1994). Bioerosive systems have also been designed (Heller, 1985; Heller et al., 1990) and pump systems described in which a pH-dependent swellable hydrogel in combination with glucose oxidase controls the insulin release (Siegel and Firestone, 1990). Yet another approach has utilized the pH-dependent solubility of an insulin derivative combined with the glucose-glucose oxidase reaction to achieve a glucose-dependent insulin release (Fischel-Ghodsian et al., 1988). More recently, a potential insulin delivery system based on glucose triggered pH-sensitive liposomes, which contain insulin and glucose oxidase, was proposed (Kim et al., 1994).

A previously unconsidered possibility for varying the release of active substances from delivery systems is the use of biosystems with microorganisms or the relevant enzymes of microorganisms to control release. In these dosage forms the activity of the biosystem is substrate-dependent. Drugs or physiologically relevant substances may enhance or reduce the activity of the biosystem. When the activity of the biosystem is associated with the production of mechanical energy, for example with production of a gas, this may be used to vary the release of a drug.

2. Materials and methods

2.1. Materials

The following substances were obtained from the sources indicated: insulin for external insulin pumps (H-Tronin 100 for H-Tron Hoechst, Hoechst, Frankfurt/M., Germany); anhydrous D-(+)-glucose for biochemistry (Merck, Darmstadt, Germany), sodium chloride (Merck, Darmstadt, Germany), fractionated coconut oil (Miglyol 812, Hüls, Witten, Germany); dried baker's yeast (Ruf Trockenbackhefe, Ruf, Quakenbrück, Germany); septum (of ethylene propylene terpolymer, one side coated with polytetrafluoroethylene; EPDM washers for screw caps, red GL 14, Schott Glaswerke, Mainz, Germany); laboratory grease (Glisseal, Borer Chemie, Zuchwil, Switzerland).

2.2. Pretreatment of the yeast

One part by weight commercial dried yeast was added to two parts of isotonic NaCl solution. This suspension was incubated for 6 h at 37°C and then freeze-dried (Lyovac GT 2, Leybold-Heraeus, Cologne, Germany).

2.3. Description of the insulin pumps

Experimental insulin pumps were constructed for these investigations which essentially consist of an insulin reservoir that can be emptied by a piston, a connecting section and an exchangeable reaction vessel (bioreactor) (Fig. 1): Freeze-dried yeast and glucose-containing solution are incubated in the bioreactor. The resulting carbon dioxide, which is dependent on the glucose concentration, is passed via an injection needle through a septum into the gas chamber behind the piston of the insulin reservoir. The pressure

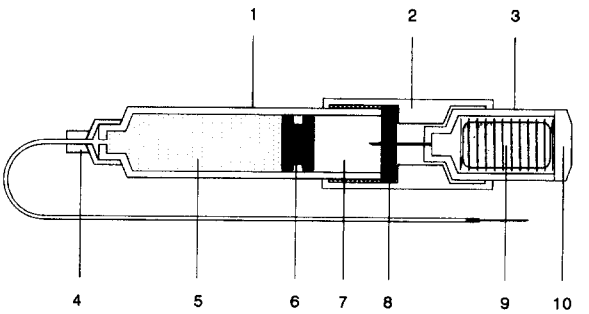

Fig. 1. Experimental insulin pump with glucose-controlled release (septum system with exchangeable bioreactor). 1, delivery unit; 2, connector section; 3, bioreactor; 4, infusion set; 5, insulin reservoir; 6, piston; 7, gas chamber; 8, septum; 9, pretreated yeast; 10, screw cap.

of the carbon dioxide moves the piston of the insulin reservoir. When the bioreactor is changed, the septum becomes airtight.

The volume of the insulin reservoir is 400 μl. It is made from the 36.1 mm long front portion of an insulin syringe (polypropylene syringe, external diameter 6.6 mm, internal diameter 4.7 mm; Omnifix-F 1 ml, Solo, Braun, Melsungen, Germany) and is fitted with an external thread (7 mm) onto which the connector section is screwed to attach the bioreactor and the septum. The connector consists of a 31.8 mm long aluminium tube (external diameter 8.6 mm, internal diameter 6.6 mm), with an inner thread (7 mm). Inside the tube is a narrowed section 10.9 mm long, 4.7 mm internal diameter, which forms a support for the septum and a guide for the needle of the bioreactor. An infusion set is attached to the front end of the insulin syringe (infusion set, 27 cm without butterfly, Disetronic Medical Systems, Sulzbach/Ts., Germany).

At the start of the experiment, the septum and piston are separated by a distance of 5.5 mm. The space between them is filled with fractionated coconut oil. The bioreactor consists of the 27.2 mm long front section of a commercial insulin polypropylene syringe with integrated injection needle (Primo-Insupak 2000; 1 ml; Büttner-Frank, Erlangen, Germany) with an external diameter of 6.6 mm and an internal diameter of 4.7 mm. The end of the syringe section is fitted with a thread onto which is screwed a cap forming an airtight seal for the bioreactor. The connection between the bioreactor and the space between the piston and the septum filled with fractionated coconut oil is made on starting up the bioreactor by piercing the septum with the injection needle. Each time the bioreactor is changed, the needle is withdrawn and the septum forms an airtight seal. The complete system without the infusion set is some 9 cm long.

2.4. Release experiments

After the insulin reservoir has been filled with 400 μl (40 IU) insulin solution for insulin pumps and the space between the piston and the septum with 100 μl fractionated coconut oil, the septum is placed in position and the insulin reservoir and the connector section are screwed together. The infusion set is attached to the insulin reservoir. 50 mg pretreated yeast are uniformly distributed on a 30 mm × 35 mm sized piece of cellulose, which is then rolled up (length: 3 cm), surrounded by a metal spiral (steel wire spring; length, 3 cm; external diameter, 4.5 mm) and introduced into the bioreactor. The yeast is moistened with 150 μl of the chosen glucose solution containing between 100 and 400 mg glucose per 100 ml and then the bioreactor immediately closed with the screw cap. The bioreactor is inserted into the connector section and the septum thereby pierced. The carbon dioxide produced in the bioreactor passes through the needle into the space between septum and piston of the insulin reservoir. The bioreactor is replaced at fixed time intervals with a new one containing freshly pretreated yeast and 150 μl of the respective glucose solution. All screw threads are lubricated with laboratory grease. Investigations of glucose-controlled insulin release were carried out at room temperature (20°C). The amount of insulin released was determined from the measured movement of the piston. The mean and standard deviation of five experiments were calculated.

3. Results

In the present studies an insulin delivery system was developed in which the release of insulin was achieved using yeast cells as the biosystem. The mechanical energy required for the glucose-dependent emptying of the insulin reservoir is provided by the carbon dioxide formed on the interaction of glucose with yeast cells. 2 mol carbon dioxide are produced from 1 mol glucose. Theoretically, if 150 μl solutions are used containing 100, 200, 300, and 400 mg glucose per 100 ml, then 37, 75, 112, and 149 μl carbon dioxide would be produced. Since commercial dried yeast contains residues of fermentable carbohydrates, the yeasts were pretreated by incubation for 6 h with isotonic saline and then freeze-dried. When yeasts subjected to this pretreatment were incubated with carbohydrate-free aqueous solutions,

virtually no fermenting activity was observed over a period of 2 h.

The design of an insulin pump with an exchangeable bioreactor is shown in Fig. 1. A commercially available insulin solution for drug pumps is contained in the insulin reservoir. A piston separates the insulin reservoir from the connector section. The exchangeable bioreactor is connected to the delivery unit via a septum. At given intervals the bioreactor is replaced by a new one, always containing 50 mg freeze-dried yeast and 150 μl glucose solution. The cellulose in which the yeast is distributed completely absorbs the glucose solution, so that the needle does not become clogged. The metal spiral surrounding the cellulose ensures the carbon dioxide can be readily discharged.

The insulin release from the insulin pump is shown in Fig. 2. A solution containing 400 mg glucose/100 ml was placed in the reactor for the first 2 h, followed by new bioreactors containing 100 mg glucose/100 ml for 1 h, 400 mg/100 ml for 2 h, and finally 100 mg/100 ml for 1 h. The results of the insulin release over 6 h show that the kinetics are directly affected by the glucose concentrations. Steady release of about 10 IU of insulin over the first 2 h is followed by a plateau without insulin release when the concentration of glucose is lowered. Insulin was released once more after the glucose concentration was increased to 400 mg/100 ml. The release stops

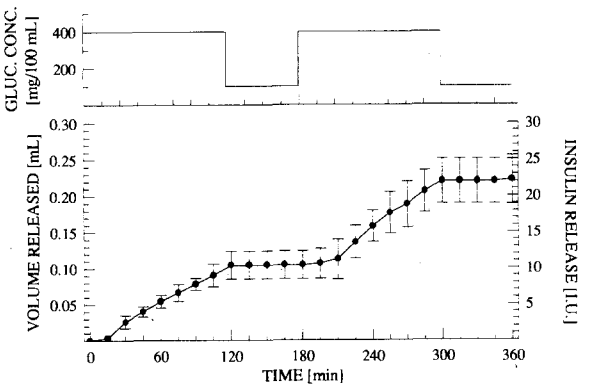

Fig. 2. Glucose-controlled insulin release from experimental insulin pumps (septum system with exchangeable bioreactor; $n = 5$, mean $\pm$ SD).

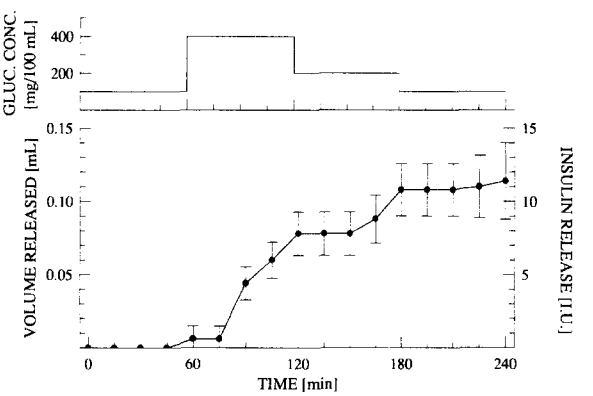

Fig. 3. Glucose-controlled insulin release from experimental insulin pumps (septum system with exchangeable bioreactor; $n = 5$, mean $\pm$ SD).

again with a concentration of 100 mg glucose/100 ml.

If a different glucose profile is used with a low starting concentration of 100 mg glucose/100 ml, followed by 400, 200, and 100 mg glucose per 100 ml, then an insulin release profile is obtained which is largely determined by these glucose concentrations. As shown in Fig. 3 the release of insulin starts when the glucose concentration is 400 mg per 100 ml. The release is reduced when a concentration of 200 mg/100 ml is used.

4. Discussion

In the present investigations a new glucose-controlled insulin delivery system was developed. By using yeast cells as a biological control mechanism, the sensing unit for the measurement of the glucose concentration and the pump mechanism with the creation of the mechanical energy required for the forward movement of the piston can be accommodated in an extremely small space compared with an electronic feedback-controlled delivery system.

The insulin delivery systems developed in the present studies are experimental systems devised as a part of fundamental research into the possibilities and limitations of a closed loop control of drug delivery using biosystems. Our research group is currently engaged in developing

glucose-controlled insulin releasing pumps based on isolated yeast enzymes. By using enzymes, it should be possible to avoid introducing live microorganisms into delivery systems. The possibility of activating systems by blood or serum samples is also under investigation. It may be possible to design insulin systems for clinical use that are activated at a certain time interval by prepared blood samples of the patient.

Acknowledgements

The present investigation is part of a dissertation in preparation (C.W.).

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