A New Route of Drug Administration: Intrauterine Delivery of Insulin and Calcitonin

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High molecular weight drugs in general, and peptides in particular, are usually delivered by parenteral route because they are poorly absorbed or degraded in the gastrointestinal tract. To optimize therapy, it is desirable to search for nonparenteral routes of administration and to deliver the drug in a controlled-release fashion. We report here on the absorption and the systemic biological effect of two peptides, insulin and calcitonin, after instillation into the uterus of the rat. Intrauterine delivery was compared to subcutaneous injections in intact and ovariectomized rats. In addition, we describe results of a preliminary study on calcitonin absorption from controlled-release matrices inserted in the rat uterus. The amount and duration of the hypoglycemic and the hypocalcemic effects induced by intrauterine delivery of insulin and calcitonin, respectively, were equivalent to those obtained after subcutaneous injections. The results were similar in intact and ovariectomized rats. It is concluded that the intrauterine administration of both insulin and calcitonin is bioequivalent to subcutaneous injection. The therapy of a number of clinically important diseases could benefit from this discovery.

KEY WORDS: drug administration; drug delivery; absorption; controlled release; drug implants; peptides; intrauterine; calcitonin; insulin.

INTRODUCTION

Clinically, the most common and accepted way for delivering drugs having relatively low molecular weights (MW; 400 to 600 Da), is the oral route. High MW drugs and very polar drugs, in general, and peptides/proteins, in particular, are usually delivered by parenteral routes because they are poorly absorbed (low permeability) or extensively degraded (metabolized) in the gastrointestinal tract. Long-term, repeated injections are often required because of the drug’s short half-life and the chronic nature of many diseases. Parenteral therapy is inconvenient, requires medical supervision, and may result in poor patient compliance. To optimize therapy, it is desirable to search for nonparenteral routes of drug administration and, for many applications, to deliver the drug in a controlled-release (i.e., continuous or pulsatile) fashion.

In order to overcome some of the drawbacks associated with parenteral administration, considerable attention has been focused on the development of transmucosal, rectal, nasal, buccal, ophthalmic, transdermal, and vaginal routes of administration (1,2). However, each of the routes mentioned above has inherent limitations such as proteolytic inactivation, low tissue permeability, and poor drug stability. Furthermore, some routes are poorly tolerated, inconvenient, and limited in application to a small number of drugs. For example, vaginal administration has been extensively studied for systemic drug administration (3). However, the vaginal bioavailability of peptides and proteins is low and too variable to be useful clinically (3). There is, therefore, a need for the development of new routes of drug administration for drugs with low oral bioavailability, such as peptide/proteins drugs.

We propose here a new route for drug administration, i.e., intrauterine drug delivery. We discovered (4) that high MW drugs such as insulin (6000 Da) and calcitonin (3450 Da) are absorbed in biologically active form from the uterus of rats. Our method may be suitable for postmenopausal women without menses. In this paper we describe the absorption and the systemic biological effect of two peptides, insulin and calcitonin, from uterus of rats. Intrauterine (iu) delivery was compared to subcutaneous (sc) injections in intact and ovariectomized rats. In addition, we describe here insulin and calcitonin absorption and biological effects following iu and sc implantation of controlled-release devices.

MATERIALS AND METHODS

Materials

Human insulin (Actrapid HM, Novo, Bagavaard, Denmark), salmon calcitonin (Mialcic, Sandoz, Switzerland), and human calcitonin (Ciba-Geigy, UK) were used. Nonradio-labeled and 14C-radio-labeled disodium hydroxyethylidene bisphosphonate (HEBP; sp act 48.9 mCi/mol) were obtained from Prof. R. J. Levy (5). Pellethane 80 AE (Dow Chemicals) was used for the preparation of drug delivery matrices. All other materials were of analytical grade.

In Vitro Diffusion Studies

The permeability of the rat’s uterus to model drugs was studied in vitro using excised uteri of intact and ovariectomized rats (30 days postovariectomy; see below). A 100-μL solution of the model drug was instilled in the lumen of each uterine horn, by a blunted 21-G, 1.5-in. syringe inserted through the vagina of the excised uterus. The drugs examined were insulin (200 μU/mL), methylene blue hydrochloride (10%, w/v), and HEBP (20%, w/v). Methylene blue and HEBP were selected as model drugs since they are positively and negatively charged molecules, respectively, at physiologic pH. In addition, the tissue staining by methylene blue facilitates easy detection of the drug. The cervix was ligated with surgical suture and each uterus was immersed in 100 mL of Kreb’s Ringer buffer (bubbled for 10 min with 95% oxygen/5% CO2). The buffer solution was stirred with a magnetic stirrer and was sampled (50 μL) at specified time points (n = 8 in each group). Insulin concentration was determined by the RIA kit (see below), and methylene blue by UV/vis spectrophotometry. HEBP concentration was deter-
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mined by combining 50 μL and 2.5 mL of scintillation fluid (Atom light, NEN, Boston, MA) and counting the mixture for carbon-14 activity in a Beckman LS 3801 liquid scintillation counter (Beckman Instruments, Berkely, CA), with reference to a calibration curve. Counting efficiency was determined from a series of quenched Beckman standards and identical control solutions.

Intrauterine Delivery—Surgical Procedures

For the iu delivery studies, a blunted syringe (21 G, 1.5 in.) was inserted through the vagina of ether-anesthetized rats into the uterus. The exact location of the needle tip in the uterus lumen was monitored through an abdominal incision. Special care was taken not to injure the uterus. Drug (HEBP, insulin, or calcitonin; see below) or saline was instilled as equally divided volumes (0.1 mL) into the two horns of each uterus. The syringe was removed and the abdominal incision was closed with surgical staples.

In some experiments [0.4 U/kg insulin study and in 10 animals (sc and iu) in the 0.8 U/kg calcitonin study], the cervix was ligated with surgical sutures in order to assure no leaks of the administered solution to the vaginal region. In another experiment (0.8 U/kg calcitonin), the uterus was ligated at the cervix and at the distal end of the uterine horns (in the oviduct/uterus region) in order to assure no leaks of the administered solution to the oviducts/ovaries/peritoneum. No ligations of the uteruses were performed in further experiments since no differences in serum drug concentrations or biological effects were observed between ligated and nonligated animals. Blood samples were collected from the tail artery under mild anesthesia at specified time points and analyzed for insulin, glucose, calcium, and calcitonin (see below). In most of the insulin delivery studies the rats were fasted overnight prior to sampling (see below). In order to avoid chronobiological effects, treatments and sampling were performed during the same time period each day (morning).

Insulin Administration

Female Sabra rats (Faculty of Medicine, The Hebrew University of Jerusalem, Israel) weighing approximately 250 g were used. For iu absorption studies with insulin, three experimental groups were used: (i) iu administration of insulin, (ii) iu administration of saline, and (iii) sc injections of insulin. A solution (0.2 mL) containing insulin (0.4 and 4 U/kg body weight) was instilled in equally divided volumes into the two horns of the uterus. Saline was instilled instead of insulin in the control group. The positive control group received the same dose of insulin as above subcutaneously. Sampling for serum insulin and glucose was performed at specified time points as described above. In the 0.4 U/kg dose study, the rats were fasted over night. Serum glucose and insulin levels were measured by glucose oxidase determination and radioimmunoassay (Sorin, Italy), respectively (6).

Controlled-Release Administration of Insulin in Diabetic Rats

Experimental diabetes was induced in female rats (n = 17; 200 g) by daily iv injections of streptozocin solution for 3 days (freshly prepared to avoid decomposition, 50 mg/kg in 0.01 M sodium citrate buffer, pH 4.5). Five days later the biological effect of controlled-release insulin was studied by inserting a sustained-release insulin implant (Linplant, Linshin, Scarborough, Canada) into the uterus using a 12-G blunted needle by the same procedure as above. These pellets release 2 U/day, and the iu delivery was compared to that in rats with subdermal implants. The control group (untreated) was sham-operated. Blood sampling was performed, as detailed above, following an overnight fast.

Calcitonin Administration

Intact (same as above) and ovariectomized rats were used in this study. Ovariectomized rats were used in order to examine drug absorption from the atrophied uterus (7). The ovaries of female rats weighing 200 g were excised under ether anesthesia ("ovariectomized"). One month postoperation the ovariectomized and intact animals' groups were randomly divided into three subgroups and treated as follows: (i) sc injections of salmon calcitonin, (ii) iu administration of salmon calcitonin, and (iii) iu administration of saline. The salmon calcitonin dose in both iu and sc groups was 0.8 U/kg body weight (0.2 mL). The procedures for the iu administration of salmon calcitonin solution and blood collection were the same as detailed in the insulin delivery experiments. Serum calcium concentration was determined by atomic absorption spectroscopy (8).

Controlled-Release Administration of Calcitonin

Two types of drug delivery devices were examined: mini osmotic pumps (ALZET 2001, Alza Corp., Palo Alto, CA), and polyurethane matrices (9,10). The mini osmotic pumps were filled with human calcitonin solution delivering 1 U/kg/day for 14 days. Polyurethane (Pellethane 80AE, Dow Chemicals) matrices containing 2% (w/w) human calcitonin and 15% (w/w) polyethylene glycol 3000 were casted on glass petri dishes from a 10% (w/v) solution in dimethylacetamide as described previously (9,10).

A small incision was made just above the cervix in the right horn of anesthetized ovariectomized rats (see above). Anesthesia was induced by intraperitoneal injection of 0.25 mL/100 g body weight of a solution containing 12.75 g chloral hydrate, 3.6 g pentobarbital, 6.5 g MgSO4, 23 mL ethanol (95%), 108 mL double-distilled water, and 108 mL propylene glycol. A matrix 1.5 x 1 x 1 cm and 0.156 ± mm thick was rolled into a cylinder shape and was inserted into the right horn of the uterus. The wound was closed with medical glue (Histacryl blau, B. Braun, Melsungen, Germany). The control group consisted of sham-operated animals. At specified time points serum human calcitonin levels were measured with a radioimmunoassay kit (ELISA-hCT, CIS Biointernational, France), and the background levels in the sham-operated rats were treated as "blank" for the calibration curve.

For the mini osmotic pumps study, another group of ovariectomized rats was divided into two subgroups: iu and sc administration. In the iu delivery subgroup one mini osmotic pump was implanted subdermally and a plastic cannula connected to the device tip was inserted through the cervix to the uterus. In the second subgroup one device was