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COMMUNICATION

Human Insulin Diffusion Profile Through a Layer of Caco-2 Cells

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ABSTRACT

The Caco-2 human colon adenocarcinoma cell line undergoes a spontaneous differentiation in culture to enterocytic cells. The diffusion of human insulin through a layer of the enterocytic differentiated Caco-2 cell line was studied using a polycarbonate membrane as a mechanical support. Human insulin (100 U) was dissolved in Dulbecco's Modified Eagle's Medium (DME) and placed on the apical side of the cells grown in culture, and allowed to diffuse under sink conditions for a period of 24 hr (37°C, pH = 7.4). Samples were collected from the receiver side at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 24 hr. The samples were then analyzed for insulin content using an HPLC method. The calculated apparent permeability coefficient (P_{app}) for human insulin through the cells was (6.5 ± 1.2) × 10^{-7} cm/sec, mean ± SE, n = 8. We conclude that using a Caco-2 cell line to study the diffusion of insulin through the intestinal barrier may be an alternative to other in vivo or in situ methods.

INTRODUCTION

Human insulin, from recombinant DNA technology, is a polypeptide drug used in the management of diabetes. Due to its chemical nature, the only route of administration currently available commercially is the parenteral route. It is desirable to determine if a route of administration such as the oral route may be used to reduce problems in bioavailability of insulin, in accurate preparation and delivery of the insulin dose, and to eliminate the discomfort of daily injections.

To examine the bioavailability of human insulin after oral administration, a whole animal (e.g., rat), in situ administration, or everted intestine model may be utilized. Another possible approach is to study the diffusion of insulin in cell culture.

The Caco-2 human colon adenocarcinoma cell line undergoes a spontaneous differentiation in culture to enterocytic cells (1). Many of the characteristics associated with normal intestinal cells are expressed by the Caco-2 cell differentiated cell line (2). These characteristics include (i) lipoprotein synthesis and secretion (3,4);

(ii) presence of sucrose-isomaltase, lactase-phlorizin hydrolase, aminopeptidase N, and dipeptidylpeptidase IV (5); and (iii) expression of intestinal peptide receptors (6).

It was found that Caco-2 can be cultured on nitrocellulose filters (7) and polycarbonate membranes (8); the latter were shown to be a better mechanical support for Caco-2 in diffusion studies (8).

MATERIALS AND METHODS

Materials

Human insulin (Humulin® R, Eli Lilly and Company (Indianapolis, IN; 100 U/ml) was used in all experiments. Caco-2 human colon adenocarcinoma cell line was purchased from American Type Culture Collection (Rockville, MD). Transwell™ clusters, polyvinylpyrroladine-free (24.5 mm in diameter, 4.71 cm^2 in surface area, and 0.4-μm pore size) were obtained from Costar (Bedford, MA). All chemicals and solvents were of analytical grade except for those used in the high-performance liquid chromatography (HPLC) procedure, which were of HPLC grade. Media for the cell culture experiments were from Sigma Chemical Company (St. Louis, MO).

Methods

Caco-2 Cell Culture

Caco-2 cell line was grown in 100 mm diameter plastic petri dishes containing Dulbecco's Modified Eagle's Medium (DME) with 10% fetal bovine serum, 1% nonessential amino acids, 1% L-glutamine, 4.5 g/liter glucose, 200 U/liter penicillin, 200 μg/liter streptomycin, 0.5 μg/liter amphotericin B, and 3.7 g/liter sodium bicarbonate, pH = 7.4. The cells grown in this medium were kept in an incubator set at 90% relative humidity in an atmosphere of 5% CO_2 (2).

Membrane Preparation

Caco-2 cells were plated in Transwell™ polycarbonate collagen-coated membranes at a density of 60,000 cells/cm^2. The culture medium and the incubation conditions were the same as stated above. Caco-2 reached full differentiation (i.e., displayed morphologic characteristics similar to those associated with normal columnar epithelium of the small intestine) after 15 days from incubation (8). These monolayers of cells maintain their integrity up to 30 days from the start of incubation (9).

Diffusion Study

All cell layers were used 19 to 21 days from incubation. On the day of the experiment, the medium on the apical side was replaced with 3 ml of solution containing 1 ml of medium and 2 ml of a dosage form (insulin solution, ghosts-insulin, vesicles-insulin, liposomes-ghosts-insulin, or liposomes-vesicles-insulin) containing 100 U of human insulin. The medium on the basolateral side was replaced with 3 ml of fresh medium only. Insulin was allowed to diffuse through the layer of cells over a period of 24 hr at 37°C. The content of the receiver side (i.e., the basolateral side) was replaced with fresh medium at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 24 hr. The same procedure was repeated using blank membranes (i.e., polycarbonate with no layer of cells.) Samples were analyzed for insulin content using HPLC (10). No interference with the insulin peak was noted by any of the components of the medium.

In another set of experiments, insulin (free or complexed with a carrier) was allowed to diffuse through a Caco-2 layer or a blank membrane. Samples were collected from the receiver side after 24 hr of diffusion and analyzed for insulin content using HPLC.

RESULTS AND DISCUSSION

The results of this study indicate that human insulin diffuses through a layer of enterocytic differentiated Caco-2 cells. The flux of insulin through this layer over the 24-hr period is presented in Fig. 1. Figure 2 represents the total amount of insulin diffused over the 24-hr period using Transwell plates. The amount of insulin diffused through the blank membrane over the 24-hr period for the carrier systems was in the order of (highest to lowest): ghosts-insulin, vesicles-insulin, liposomes-vesicles-insulin, and liposomes-ghosts-insulin. However, when the cell layer was present, the order of the amount diffused over the 24-hr (highest to lowest) was: vesicles-insulin, liposomes-vesicles-insulin, ghosts-insulin, and liposomes-ghosts-insulin. This order sequence correlated well with our finding after intraduodenal administration of a carrier-insulin suspension in streptozocin diabetic rats (r = 0.915) (11) (Fig. 3). Blood glucose concentration reached a minimum after 2 hr of the intraduodenal dose of carrier-insulin (100 U) in that study (11).

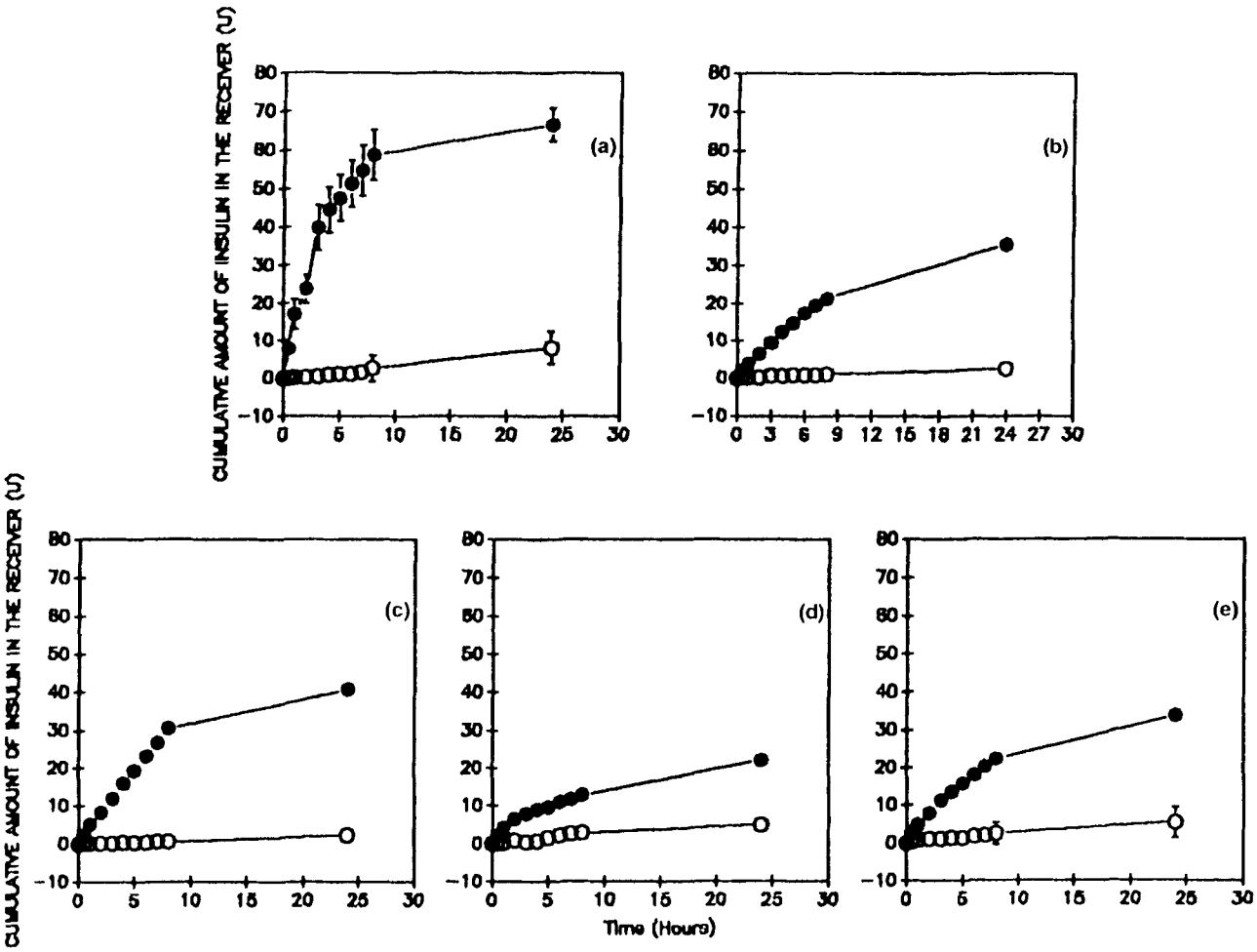

Figure 1. The diffusion profile of human insulin through a layer of differentiated Caco-2 cells (○) or a blank membrane (●). Either (a) insulin solution (3 ml, 100 U), (b) ghosts-insulin, (c) vesicles-insulin, (d) liposomes-ghosts-insulin, or (e) liposomes-vesicles-insulin was placed in the donor side of the diffusion cell. Insulin diffusion was monitored by assaying the contents of the receiver side at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 24 hr. The temperature was maintained at 37°C. Data points are the mean ± *SD* of 6 to 8 observations.

The apparent permeability coefficient (P_{app}) measures the rate by which insulin is diffused through the barrier. P_{app} of insulin from a solution was calculated from the diffusion data according to the following equations:

$$\ln(C_d/C_0) = -K\,t \quad (1)$$

$$K = P_{app}\,S/V_d \quad (2)$$

where: C_d is the concentration of insulin in the donor compartment at time t; C_0 is the initial concentration of insulin in the donor compartment; K is the first-order disappearance rate of insulin from the donor compartment = (0.0036 ± 0.0007 hr^{-1}, n = 8) (mean ± SEM, number of observations); V_d is the volume in the donor compartment = 3 ml; P_{app} is the apparent permeability coefficient; and S is the surface area of diffusion = 4.71 cm^2.

The value of K indicates that 0.36% of insulin diffuses per hour through a Caco-2 layer. Using an everted rat small intestine preparation, Schilling and Mitra (12) have shown a value of P_{app} of (7.80 ± 1.54) × 10^{-7} cm/sec for the diffusion of insulin through the distal

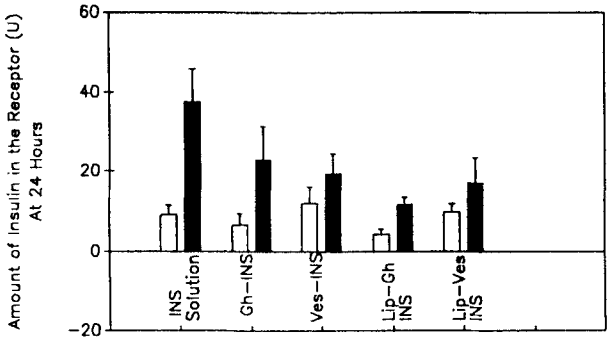

Figure 2. The amount of insulin diffused over a 24-hr period through a layer of Caco-2 differentiated cells (□) or a blank membrane (■). Free insulin solution (3 ml, 100 U) or a carrier-insulin suspension (3 ml, 100 U) was placed in the donor side of a diffusion cell. The content of the receiver side was assayed for insulin at 24 hr. The temperature was maintained at 37°C. Bars represent mean ± *SD* of 6 observations.

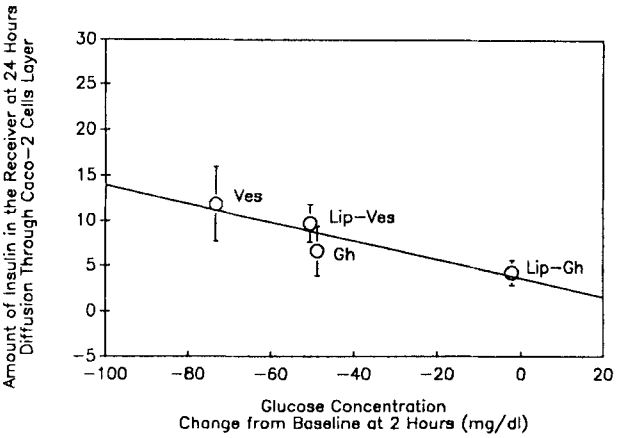

Figure 3. A very high linear correlation was found between the amount of insulin diffused through a layer of Caco-2 differentiated cells and the change from baseline of the blood glucose concentration (mg/dl) at 2-hr after intraduodenal administration of a carrier-insulin suspension in streptozocin diabetic rats. The linear equation is $Y = -0.103X + 3.61$ ($r = 0.915$). Data points are mean ± *SD* of 6 to 8 observations.

jejunum. The value for insulin P_{app} through a Caco-2 layer was $(6.5 \pm 1.2) \times 10^{-7}$ cm/sec. This is very close to that found using the everted rat small intestine.

CONCLUSIONS

The use of an enterocytic differentiated Caco-2 cell line in culture to study the diffusion of human insulin through intestinal membrane may be an alternative to other in vivo or in situ methods. Such a procedure may reduce the number of whole-animal experiments that must be used to examine factors affecting the movement of insulin or other proteins through membranes.

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REFERENCES

1. J. Fogh, M. J. Fogh, and T. Orfeo, J. Natl. Cancer Inst., 59, 221 (1977).
2. J. I. Hidalgo, A. Kato, and T. R. Borchardt, Biochem. Biophys. Res. Commun., 160(1), 317 (1989).
3. E. T. Hughes, V. W. Sasak, M. J. Ordovas, M. T. Forte, S. Lamon-Fava, and J. E. Schaefer, J. Biol. Chem., 262(8), 3762 (1987).
4. G. M. Traber, J. H. Kayden, and J. M. Rindler, J. Lipid Res., 28, 1350 (1987).
5. H.-P. Hauri, E. E. Sterchi, D. Bieng, A. M. J. Fransen, and A. Marxer, J. Cell Biol., 101, 838 (1985).
6. M. Laburthe, M. Rousset, and C. Rouyer-Fessard, J. Cell Biol., 262, 10180 (1987).
7. E. Grasset, M. Pinto, E. Dussaulx, A. Zweibaum, and J.-F. Desjeux, Am. J. Physiol., 247 (Cell Physiol. 16), C260 (1984).
8. J. I. Hidalgo, J. T. Raub, and T. R. Borchardt, Gastroenterology, 96, 736 (1989).
9. P. Artursson, J. Pharm. Sci., 79(6), 476 (1990).
10. A. Al-Achi and R. Greenwood, Drug Dev. Ind. Pharm., 19(6), 673 (1993).
11. A. Al-Achi and R. Greenwood, Drug Dev. Ind. Pharm., 19(11), 1303 (1993).
12. J. R. Schilling and A. K. Mitra, Int. J. Pharmaceut., 62, 53 (1990).