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DIFFUSION PROFILE OF HUMAN INSULIN THROUGH POLYCARBONATE MEMBRANE

A. Al-Achi and R. Greenwood
Campbell University School of Pharmacy
Buies Creek, North Carolina 27506

ABSTRACT

The aim of this study was to investigate the diffusion of human insulin through polycarbonate membrane, a common mechanical support used in cell culture studies. The pore size of the polycarbonate membranes, the presence of collagen on the surface of the membrane, and the pH of the medium affected the diffusion of insulin through the membrane. Minimum diffusion occured at pH near the isoelectric point of insulin (5.3). The larger the pore size of the polycarbonate membrane the higher the amount diffused. The transfer rate of insulin was slower when membranes were coated with collagen.

INTRODUCTION

Type I diabetes mellitus is a disease that must be managed by the use of insulin. This treatment requires injections of insulin either once daily or more frequently. Because of the discomfort associated with injections or to improve ease and acceptance of therapy, another route of administration, such as the oral route, may be desirable. In order to administer insulin orally, the drug would have to be protected from intestinal enzymes that are capable of degrading it. Introducing the drug orally in a liposome form has shown some protection, although not enough so that the liposome- insulin produced a significant reduction in blood glucose concentrations (1).

So far, studies done on the oral absorption of insulin involved the use of experimental animals, such as rats (1,2,3), rabbits (4), and dogs (5). However, the use of animals in these studies may potentially be replaced by the use of a cell culture model, such as the caco-2 human colon adenocarcinoma cell line which undergoes a spontaneous differentiation in culture to enterocytic cells (6) which then resemble normal intestinal cells (7,8,9,10). A physical support used commonly for caco-2 cells

grown in culture is collagen-coated polycarbonate membrane. The effect of this collagen or the polycarbonate membrane itself on transport has not been examined when studying drug movement through cultured cells.

In investigating possible oral dosage formulations for insulin, it has been shown that human insulin can associate with erythrocyte-membrane in the form of erythrocyte-ghosts (11,12), erythrocyte-vesicles (i.e., ultrasonicated ghosts) (12), lipid-coated-ghosts (11,12) or lipid-coated-vesicles (12). In the case of ghosts, insulin association was in the form of both adsorption on the surface of the ghosts and encapsulation within the ghosts (12).

The present study examined the use of collagen-coated polycarbonate membranes as physical supports for cells grown in culture for insulin transport studies. The diffusion of human insulin, free or attached to an erythrocyte-membrane carrier system, through collagen-coated or uncoated polycarbonate membrane was investigated.

MATERIALS AND METHODS

Materials

Human erythrocytes were from the American Red Cross, North Carolina. Human insulin (Humulin R, Eli Lilly) was obtained from N.C. Mutual, North Carolina. Lipids were purchased from Sigma Chemical Company, St. Louis, Missouri. Polycarbonate membranes were from Poretics Corporation, Livermore, California. The diffusion cell (Valia-Chien Skin Permeation System) was purchased from Crown Glass Company, Somerville, New Jersey. Chemicals and solvents that were used in the analysis of insulin were of HPLC grade. All other chemicals were of analytical grade.

Methods

1. Preparation of Erythrocyte-Ghosts

The suspension of ghosts was prepared using a method previously described (13). A volume of human erythrocytes was initially washed with an isotonic phosphate buffer solution. Hemolysis was then induced by repeated washing of the cells with a hypotonic phosphate buffer solution. The number of ghosts in the suspension was shown to be approximately 4 million per ml (13).

2. Preparation of Erythrocyte-Vesicles

The suspension of vesicles was prepared based on a method previously described (13). Five ml of ghosts suspension were ultrasonicated at an energy level of 50 to produce fragmented ghosts, known as vesicles.

3. Preparation of Liposomes-Ghosts and Liposomes-Vesicles

The suspensions of these two carrier systems were prepared according to a method previously described (11,12). A film of dry lipids [Cholesterol and L-α-phosphatidyl-choline (type XI-E from fresh egg yolk)] in a round bottom flask was dispersed with a liquid mixture containing 50% of either ghosts or vesicles suspensions and 50% of a swelling solution (containing NaCl, KCl, and CsCl). The flask was gently shaken for 1 h at room temperature and then allowed to stand overnight in the refrigerator. Following centrifugation of the mixture and separation of the supernatant, the sediment was dispersed in 5 ml of the swelling solution. The final suspension was stored at 4°C for later use.

4. Incubation of Insulin with Carrier Systems

One ml of human insulin solution (Humulin R, Eli Lilly, 100 U/ml) was incubated for 24 h at 37°C with 1 ml of either one of the four carrier suspensions described above. The amount of insulin associated with the carriers was found to be [average ± S.D., (number of samples)]: ghosts 56.12 ± 12.56 (11), vesicles 32.50 ± 15.06 (6), liposomes-ghosts 45.44 ± 6.10 (6), and liposomes-vesicles 26.63 ± 7.55 (6) (12).

5. High Performance Liquid Chromatography Method for Insulin

The HPLC column was a Dupont Instruments 10 μm 4.6 mm ODS x 25 cm C-18 reverse phase column. The mobile phase was composed of water: acetonitrile: trifluoroacetic acid: hexanesulfonic acid (65:35:0.1:0.1), with a solvent flow rate set at 1 ml/min. All measurements were carried out at a wavelength of 215 nm (12).

6. Rat Collagen Preparation

Rat collagen was used in coating polycarbonate membranes in the diffusion experiments. The collagen was extracted from four rat tails by dissecting and removing the tendons according to a method described previously (14) with minor modifications. The fibers were suspended in 600 ml of a 3 mg/ml solution of acetic acid and shaken occasionally for 48 h at 4°C. Subsequently the solution was left to stand without agitation for 48 h allowing the undissolved fibers to sediment. The mixture was then filtered through four layers of cheese cloth and centrifuged at 12,000 x g for 2 h. The supernatant was collected and precipitated with 120 ml of 30 % NaCl solution. The resulting mixture was centrifuged at 4,000 x g for 30 min. The sediment was rinsed three times with 5 % NaCl solution. After each rinse, the supernatant was aspirated and discarded. The sediment after the final wash was dissolved in 0.6 % acetic acid solution. The resulting solution was divided in several

portions (each 30 ml in volume) and each portion was dialysed against 500 ml of 1 mM HCl solution for 48 h at 4°C. Spectropore dialysis bags with a molecular weight cut off equal to 50,000 were used so that smaller molecular weight impurities were removed. The final volume of the collagen solution was about 200 ml with a concentration of collagen of 7.4 mg/ml (determined by lyophilizing 5 ml of the final collagen solution and weighing the dry residue).

7. Diffusion of Human Insulin Through Polycarbonate Membranes

All diffusion studies were conducted using a membrane permeation system with the following specifications: i) the donor and receptor chambers each contained 3.5 ml total volume, ii) the solution inside each of the chambers was continuously mixed using a teflon-coated magnetic stir bar at a constant speed of 600 r.p.m., iii) the diameter of the opening between the two chambers was 9 mm, and iv) all experiments were done at 37°C. The polycarbonate membranes were plain membranes with 0.2, 1.0, and 8 μm pore sizes; the diameter of the membrane (13 mm) was large enough to cover the opening between the two chambers.

A) Effect of pH

U.S.P. buffer solutions with different pH's were prepared: i) acid phthalate buffer pH = 2.8, ii) acid phthalate buffer pH = 4.0, iii) neutralized phthalate buffer pH = 5.0, iv) phosphate buffer pH = 7.0, and v) alkaline borate buffer pH = 9.8. The buffer solution was added to the receptor compartment of the diffusion cell (3.5 ml). One ml of human insulin solution (100 U/ml) was added to the donor compartment and enough buffer was added to bring the total volume to 3.5 ml. After 24 h, the contents of the receptor compartment were removed and analysed for insulin. For these experiments, 0.2 μm pore size polycarbonate membranes were used.

B) Effect of membrane pore size

To test the effect of the pore size on the diffusion of insulin through polycarbonate membrane, 0.2, 1.0, and 8.0 μm pore sizes were tested. Since Humulin R (Eli Lilly) has a neutral pH, pH 7.0 phosphate buffer solution (3.5 ml), was placed in the receptor compartment. One ml of the insulin solution (100 U/ml) was placed in the donor compartment and enough pH 7.0 phosphate buffer was added to bring the total volume to 3.5 ml. Insulin was allowed to diffuse through the membrane for a period of 24 h. The amount of insulin diffused through the membrane after 24 h was determined by HPLC quantitation of the insulin in the receptor compartment.

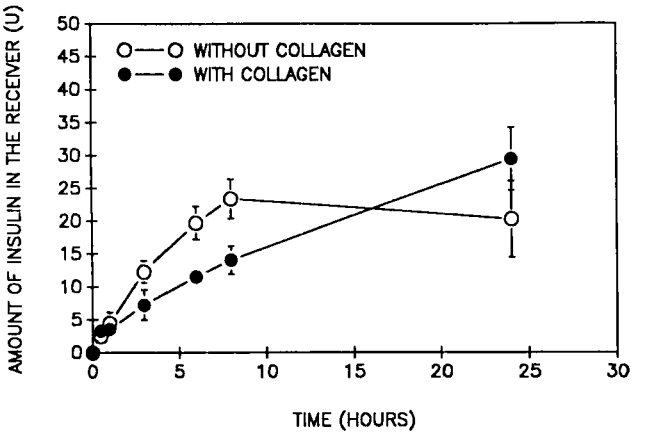

FIGURE 1

The diffusion profiles of insulin through polycarbonate membranes (0.2 μm pore size) over a 24 h period. Insulin was placed in the donor compartment of a diffusion cell in the form of a solution. The pH of both the donor and the receptor compartment was maintained at 7.00. The receptor compartment contained the phosphate buffer solution only. Data points are mean ± S.D. of 4 samples.

C) Effect of collagen

The diffusion profile of insulin through collagen-coated or uncoated polycarbonate membrane (0.2 μm) was tested. Polycarbonate membranes were coated with rat collagen by saturating both sides of the membrane with the collagen solution for a period of 1 min just prior to the diffusion experiment. One ml of insulin solution (100 U/ml) was mixed with either 1 ml of hypotonic phosphate buffer solution or 1 ml of one of the carrier systems mentioned above. The mixture was incubated for 24 h at 37°C. Following the incubation, the mixture was placed in the donor compartment of the diffusion cell, and the volume was made up to 3.5 ml with pH 7.0 phosphate buffer solution. The receptor compartment contained 3.5 ml of pH 7.0 phosphate buffer solution. The amount of insulin in the receptor compartment was determined at 0.5, 1, 3, 6, 8, and 24 h by assaying for insulin in a 100 μl aliquot removed from the receptor compartment.

RESULTS AND DISCUSSION

Polycarbonate membranes have been used in cell culture studies as support membranes for the cells (15). The diffusion of human insulin through this membrane

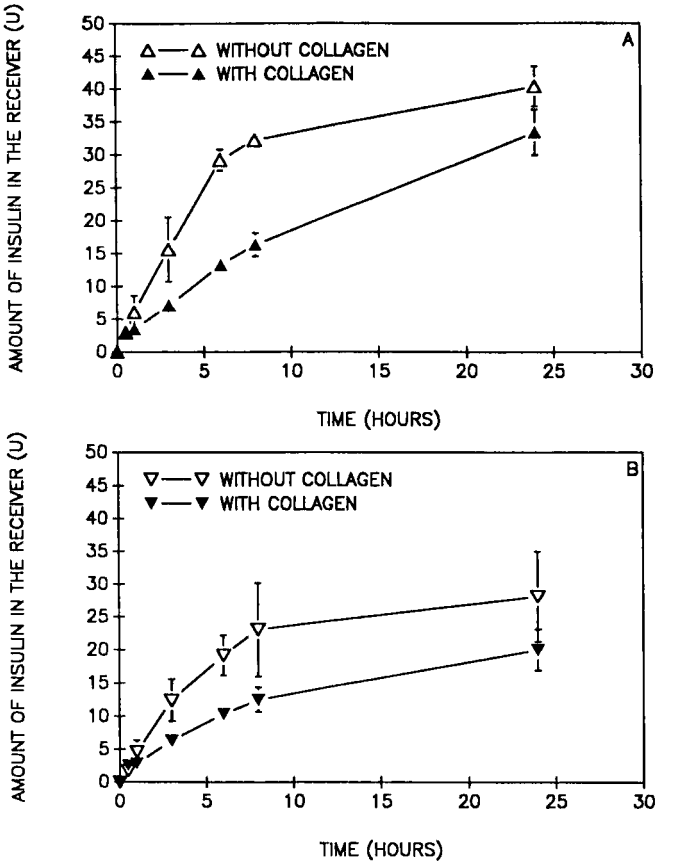

FIGURE 2

The diffusion profiles of insulin through polycarbonate membranes (0.2μm pore size) over a 24 h period. Insulin was placed in the donor compartment of a diffusion cell in the form of either A) erythrocyte-ghosts-insulin or B) liposomes-ghosts-insulin. The pH of both the donor and the receptor compartment was maintained at 7.00. The receptor compartment contained the phosphate buffer solution only. Data points are mean ± S.D. of 4 samples.

was found to be a factor of the pore size of the membrane and the pH of the environment. As expected, the diffusion of insulin reached its minimum at pH near 5.0 (i.e., close to the isoelectric point of insulin, 5.3). Diffusion was greater at lower or higher pH's, but was optimum at alkaline pH. An increase (mean ± S.D., 4 samples) in the pore size of the membrane from 0.2 μm to 1.0 μm did not result in any significant increase in the amount of insulin transferred (23.56 ± 6.08 U for 0.2 μm vs 24.51 ± 3.30 U for 1.0 μm). On the other hand the 8.0 μm pore size resulted in an

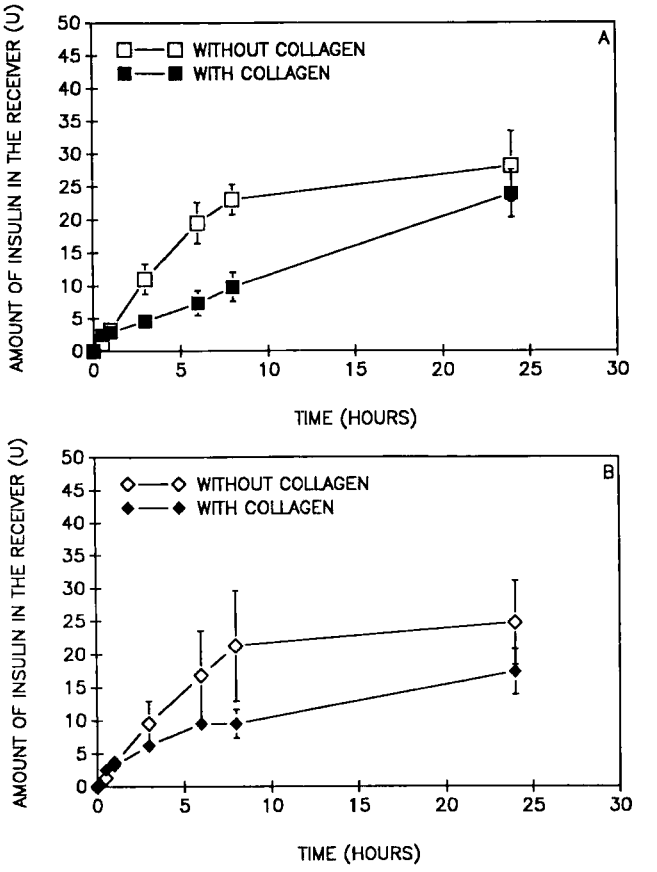

FIGURE 3

The diffusion profiles of insulin through polycarbonate membranes (0.2 μm pore size) over a 24 h period. Insulin was placed in the donor compartment of a diffusion cell in the form of either A) erythrocyte-vesicles-insulin or B) liposomes-vesicles-insulin. The pH of both the donor and the receptor compartment was maintained at 7.00. The receptor compartment contained the phosphate buffer solution only. Data points are mean ± S.D. of 4 samples.

increase in the amount of insulin diffused (30.56 ± 4.45 U, P = 0.048). However, because the smallest size was that of vesicles (about 0.2 μm) (13), the 0.2 μm pore size was chosen for the diffusion experiments to prevent possible passage of vesicles through the membrane. However, this size limitation is not necessary when larger carrier systems are used (such as the case of ghosts, liposomes-ghosts, and liposomes-vesicles).

TABLE 1
Total Cumulative Amount of Insulin (U) Diffused Through Polycarbonate Membranes Over a Period of 24 h. Initial Amount of Insulin in the Donor Cell was 100 U. Values are Ave. ± S.D. for 4 Samples.

Carrier System	Uncoated	Collagen-Coated
INS Solution	20.26 ± 5.83	29.48 ± 4.79
Ghosts-INS	40.37 ± 3.02[a]	33.40 ± 3.44
Vesicles-INS	28.08 ± 5.36[b]	23.90 ± 3.64
Liposomes-Ghosts-INS	28.17 ± 6.88	20.05 ± 3.08[c]
Liposomes-vesicles-INS	24.75 ± 6.36	17.36 ± 3.46[d]

a. Significantly larger than insulin solution (P = 0.001).
b. Significantly larger than insulin solution (P = 0.096).
c. Significantly smaller than insulin solution (P = 0.032).
d. Significantly smaller than insulin solution (P = 0.006).

Figures 1, 2, and 3 show the data obtained from the diffusion study. The results indicate that the presence of collagen on the surface of polycarbonate membrane slowed the transfer of insulin through the membrane (P values equal to 0.01 for insulin solution, 0.0001 for ghosts-insulin, 0.0001 for vesicles-insulin, 0.0001 for liposomes-ghosts-insulin, and 0.009 for liposomes-vesicles-insulin). In terms of the cumulative amount of insulin in the receptor after a 24 h diffusion period (Table 1), there was a significant increase for ghosts (P = 0.001) or vesicles (P = 0.096) (uncoated membrane), and a significant decrease for liposomes-ghosts (P = 0.032) or liposomes-vesicles (P = 0.006) (coated membranes). Thus, collagen presence not only slowed the rate of transfer but also reduced the total amount transferred over a period of 24 h when carrier systems were used.

CONCLUSION

The results of this study demonstrated the ability of human insulin to diffuse through collagen-coated or uncoated polycarbonate membrane. The influence of the pH of the medium, the pore size of the membranes, the presence of collagen on the surface of membranes, and the use of a carrier system on changing the diffusion profile of the drug should be considered when studying the transfer of insulin and potentially other drugs in cell culture studies.

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