The Gastrointestinal Absorption of Liposomally Entrapped Insulin in Normal Rats

HARISH M. PATEL and BRENDA E. RYMAN

Department of Biochemistry, Charing Cross Hospital Medical School, Fulham Palace Road, London W6 8RF, U.K.

The use of liposomes as carriers of therapeutic agents has been extensively reviewed (Tyrrell et al., 1976). We (Patel & Ryman, 1976) have shown that encapsulation within liposomes may be an effective way of administering insulin orally, by which route it is normally destroyed in the gastrointestinal tract. When liposomally entrapped insulin was given orally to diabetic rats, there was a significant decrease in blood glucose, whereas the same amount of free insulin had no effect on the blood glucose (Patel & Ryman, 1976). Thus liposomes may be protecting the degradation of the protein by proteolytic digestion and possibly facilitating its absorption in the gastrointestinal tract. However, the hypoglycaemic effect was not observed when liposomally entrapped insulin was administered orally to normal rats, contrary to the results of Dapergolas & Gregoriadis (1976). Two possibilities were considered to explain our failure (Patel & Ryman, 1976) to observe the hypoglycaemic effect in the normal rat. The effective absorption of the liposomally entrapped protein may possibly be dependent on the diabetic state of the animal. It is known that an increase in intestinal brush-border-enzyme activities occurs in the mucosa of diabetic rats (Caspay et al., 1972), and an increase in intestinal growth and the transport of hexose sugars (Schedl & Wilson, 1971) and amino acids (Younoszai & Schedl, 1972) has also been reported and could possibly explain our observations with liposomally entrapped insulin in diabetic rats. The second possibility is that the amount of intact insulin absorbed may not be sufficient to produce any significant change in the blood glucose in normal rats as it may be broken down by the liver. As much as 40–50% of insulin absorbed from the intestine is destroyed by the liver in a single transhepatic circulation (Mortimore & Tietze, 1959; Madison et al., 1959; Blackard & Nelson, 1970). In diabetic rats the absorbed insulin may not be destroyed so readily by liver, because the activity of the hepatic enzyme primarily responsible for the degradation of insulin, namely glutathione-insulin transhydrogenase (EC 1.8.4.2) is reported to be decreased in diabetic rats (Cudworth & Barber, 1975).

To investigate the above possibilities, we treated normal rats with indol-3-ylacetic acid (3 mmol/kg administered intraperitoneally), a compound known to inhibit insulin degradation (Mirsky et al., 1956), and immediately afterwards 6i.u. of liposomally entrapped insulin was administered orally. The liposome preparation used was as described earlier (Patel & Ryman, 1976) with the small alteration that phosphate buffer was replaced by 50mm-Tris/NaCl buffer, pH 7.4. Monocomponent pig insulin used was a gift from Dr. W. R. Buckett (Novo Industries, Copenhagen, Denmark).

The results in Table 1 show that 6i.u. of liposomally entrapped insulin given orally to normal rats led, perhaps surprisingly, to an apparent increase in blood glucose (123% of initial value), but the same preparation given orally to indolylacetic acid-treated normal rats markedly decreased the blood glucose (54% of initial value). This decrease is greater than that observed with indolylacetic acid followed by oral feeding of control liposomes (i.e. liposomes without insulin). These results indicate that liposomally entrapped insulin is absorbed in the gastrointestinal tract of normal rats, but the absorbed insulin requires protection against its degradation (which is probably carried out by the liver) to produce a hypoglycaemic effect in such rats. This protection is provided by indolylacetic acid, probably by inhibiting hepatic glutathione-insulin transhydrogenase activity. We could not observe any decrease in blood glucose in normal rats given 2.5–5i.u. of insulin entrapped in dipalmitoyl phosphatidylcholine/cholesterol liposomes as used by Dapergolas & Gregoriadis (1976).

During these studies, we have observed that not all preparations of liposomally entrapped insulin produce a hypoglycaemic effect in either diabetic or indolylacetic acid-treated normal rats. At present we suspect that insulin might be denatured in...
Table 1. Hypoglycaemic effect of orally administered liposomally entrapped insulin in indol-3-ylacetic acid-treated normal rats

Normal rats (body weight 125–160g) were injected intraperitoneally with 3 mmol of indolylacetic acid/kg and immediately afterwards liposomes (50–60 mg of lipid), with or without 6 i.u. of insulin, were administered by the oral route. The blood samples were taken immediately before and 1 h after liposome administration. The results are expressed as the mean ± S.E.M. of the percentage of initial blood glucose, which was 95–105 mg/100 ml of blood.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>No. of rats used</th>
<th>Blood glucose (% of initial value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Indolylacetic acid alone</td>
<td>4</td>
<td>88 ± 1</td>
</tr>
<tr>
<td>(2) Indolylacetic acid + liposomes without insulin</td>
<td>11</td>
<td>88 ± 6</td>
</tr>
<tr>
<td>(3) Indolylacetic acid + liposomes + free insulin (6i.u.)</td>
<td>4</td>
<td>126 ± 9</td>
</tr>
<tr>
<td>(4) Liposomally entrapped insulin (6i.u.) alone</td>
<td>4</td>
<td>123 ± 12</td>
</tr>
<tr>
<td>(5) Indolylacetic acid + liposomally entrapped insulin (6i.u.)</td>
<td>8</td>
<td>54 ± 8</td>
</tr>
</tbody>
</table>

The presence of lipid during these ‘faulty’ preparations. We have also observed that with a given preparation of liposomally entrapped insulin the hypoglycaemic effect varies from animal to animal, and one or two animals in a group often failed to show any change in blood glucose. This results in the high S.E.M. seen in Table 1, and suggests that other factors such as the nutritional state of the animal and stress should also be considered.

Phosphatidylinositol-associated insulin has been claimed to produce a hypoglycaemic effect in normal rats (Dapergolas et al., 1976). However, we have observed that phosphatidylinositol alone, when given orally (40 mg/kg) to normal rats, decreases blood glucose by 15–20% of the initial value. This value is comparable with that reported by Dapergolas et al. (1976) for phosphatidylinositol-associated insulin. Moreover the hypoglycaemic effect of phosphatidylinositol was not observed in streptozotocin-diabetic rats.

The mechanism of absorption of liposomally entrapped protein in the gastrointestinal tract remains unknown. Dapergolas & Gregoriadis (1976) have claimed that intact liposomes are identified in the circulation after oral administration. However, in preliminary experiments we have failed to detect the presence of gentamicin, a non-absorbed antibiotic, in the blood in the presence or absence of Triton X-100 after oral administration of liposomally entrapped gentamicin. If liposomes are absorbed intact it might have been expected that the antibiotic would be detected by the sensitive bacteriological technique used.

We gratefully acknowledge the financial support of the British Diabetic Association.


Vol. 5