ORAL ADMINISTRATION OF INSULIN BY ENCAPSULATION WITHIN LIPOSOMES

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1. Introduction

It is generally accepted that for therapeutic use, substances such as peptides (and certain drugs) cannot be given orally to patients as they will be destroyed by the digestive juices. Insulin, for example, can only be given effectively by injection because it is degraded by proteolytic digestion in the gastro-intestinal tract when taken orally. This is a problem for diabetic patients who are unable to inject their insulin themselves. Hence there is a need for an effective mechanism to allow administration of such proteins orally without losing their biological activities. Insulin as insulin hydrochloride mixed with a vasodilator in a tablet form has been successfully administered by allowing the tablet to be absorbed under the tonque [1]. However in this method the long-term effect of large doses of an unphysiological concentration of the vasodilator employed is questionable.

The use of liposomes as carriers of therapeutic agents such as enzymes (in enzyme replacement therapy), drugs, chelating agents and cell modifying compounds has been recently discussed [2]. Liposomes are spherules formed when phospholipids are allowed to swell in aqueous media and they consist of concentric closed lipid bilayers alternating with aqueous compartments. Within the aqueous phase of liposomes, water-insoluble substances can be entrapped [3]. We thought that substances like insulin could possibly be administered orally without loss of biological activity if protected from the digestive juice by encapsulation within liposomes.

The preliminary experiments reported here demonstrate the possibility of oral administration of insulin by such encapsulation. When liposomes containing entrapped insulin were given orally to diabetic rats, there was a significant reduction of the blood-glucose level, whereas the same amount of free insulin had no effect on the blood-glucose level.

2. Materials and methods

Egg phosphatidylcholine (lecithin) was purchased from Lipid Products (Epsom); dicetyl phosphate from K. and K. Laboratories. Cholesterol and crystalline insulin from bovine pancreas were from Sigma. Glucose oxidase (Grade I) and horseradish peroxidase (Grade I) were obtained from Boehringer and *o*-dianisidine dihydrochloride from BDH. Streptozotocin was a gift from Dr K. Rookledge. [^{125}I]insulin (5 μCi/0.1 μg) was purchased from Radiochemical Centre, Amersham. All other reagents were of analytical grade.

Liposomes were prepared by dissolving lecithin, cholesterol and dicetyl phosphate in a molar ratio of 10:2:1 in chloroform [4]. The lipid mixture was dried on a rotary evaporator at 37°C. Insulin

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(2.0 mg/ml) suspended in 5 mM phosphate buffer, pH 7.2 together with a trace of ^{125}I-iodinated insulin was allowed to stand for 5 min and the supernatant was added to the dry lipid and gently shaken under N_2. (A total of 2 mg of insulin was used for each 30 mg of lipid). The liposomes thus formed were allowed to stand at room temperature for 1 h and then sonicated for 1 min at 4°C using a 19 mm titanium probe at 1.5 A in an MSE 150W sonicator. The suspension was then kept at 4°C for a further hour and then centrifuged in an MSE Superspeed 50 centrifuge at 120 000 *g* for 3 h. The liposomal pellet was resuspended in the phosphate buffer and centrifuged once again as described above. Finally the pellet was suspended in a minimum amount of the phosphate buffer and just before use was diluted with the buffer to give a final concentration of liposomes of 60–70 mg lipid/ml. For control experiments liposomes containing no insulin were prepared in the absence of the protein and this preparation was mixed with insulin solution just before use. Percentage entrapment (including any possible non-specific associated protein with liposomes) [5] was measured by assaying ^{125}I-radioactivity associated with liposomes in a Wallac γ-counter (GTL 300–1000). The percentage of 'total' entrapment of insulin varied (from 6 to 26% of the initial amount of insulin used) with the batch of insulin and from preparation to preparation of liposomes. The insulin was not re-assayed but its activity shown here was based on that given by Sigma Chemicals Ltd., namely 24 IU per mg protein.

Male and female albino rats (Wistar) were used indiscriminately and were made diabetic by intravenous injection of streptozotocin (6.5 mg/100 g body wt.) [6]. The rats were used within 1–4 weeks after streptozotocin injection. They were checked to be diabetic by examining the glucose level in their urine. The liposomes containing insulin were administered to the diabetic or normal rats either orally or intraperitoneally. For control experiments, either free insulin or insulin + liposomes not containing protein (i.e. control liposomes) were similarly administered. Blood samples were taken either from the tail or by heart puncture before and 3 h after the administration of insulin. Blood glucose was determined enzymatically using glucose oxidase [7,8]. The blood-glucose level in the diabetic rats was greater than 400 mg/100 ml of blood, and that in normal rats was between 90–110 mg per 100 ml of blood.

Table 1
Comparison between the effects of oral and intraperitoneal administration of entrapped or non-entrapped insulin on the blood-glucose level of diabetic rats

	Units of insulin administered			
	Orally		Intraperitoneally	
	5 IU	12 IU	5 IU	12 IU
	Blood-glucose content as % of initial level (Mean value ± standard deviation)			
Free insulin	97.5 ± 8.8	85.8 ± 15.4	21.1 ± 5.4	10.6 ± 3.3
Liposome-entrapped insulin	63.4 ± 14.2	40.7 ± 16.5	24.0 ± 6.2	20.7 ± 4.5

Diabetic rats (body weight 175–100 g) were given entrapped or free insulin either orally or intraperitoneally. Each rat received 60–70 mg lipid (as liposomes) in 1 ml phosphate buffer. Blood samples were taken before and 3 h after insulin administration. The results are expressed as the percentage of the initial blood-glucose level (i.e. before insulin administration). Five rats were used for oral study and three for intraperitoneal injection.

3. Results and discussion

Oral administration of insulin (12 IU) entrapped within liposomes reduced the blood-glucose level in diabetic rats to about one-third of the initial value by 3 h after administration. These results are comparable with those observed when the same amount of entrapped insulin was administered intraperitoneally (table 1). However the picture is different when the same amount of free insulin (12 IU) either alone (table 1) or with control liposomes (not shown) is administrated orally or intraperitoneally. When given orally to diabetic rats, free insulin alone, or insulin given with liposomes (but not entrapped), rats failed to reduce the blood-glucose level by any significant amount and the values were not comparable with those obtained when the free insulin was administered intraperitoneally (table 1). However the blood-glucose level was found to decrease to about 50% of the initial value if the oral dose of free insulin was increased to 96 IU. The results show that liposome-entrapped insulin retains its activity after being absorbed in the gut, whereas free insulin is only effective when administered orally in large unphysiological quantities. Thus insulin when encapsulated within liposomes is probably protected from proteolytic degradation in the gastrointestinal tract.

Insulin is known to bind to phospholipids under certain conditions [9] and could possibly be electrostatically associated with the surface of liposomes [5] rather than being entrapped within liposomes. However the results discussed above discard the possibility that insulin is protected from proteolytic digestion in the gastrointestinal tract by its nonspecific association with phospholipid, as free insulin mixed with the control liposomes had the same lack of effect on the blood-glucose level as did free insulin alone.

The effect of insulin on the blood glucose level was studied mainly 3 h after its oral administration. The results of preliminary experiments indicate that liposomally entrapped insulin is probably absorbed into the circulation within one hour of its oral administration. This was judged by the reduced blood-glucose level observed within one hour of the oral dose of liposome-entrapped insulin. The amount of lipid administered orally as liposomes (50–70 mg, containing no protein) has no effect on the blood-glucose level in diabetic rats.

When 12 IU of liposome-entrapped insulin was orally administered to normal rats, no change in the blood glucose level was observed (table 2) as compared with a lowering of blood-glucose when the same amount of free insulin was given intraperitoneally. It was moreover observed that in a few rats which were previously injected with streptozotocin, but whose blood-glucose levels were not markedly elevated (due to unknown reasons), the oral administration of 12 IU of liposome-entrapped insulin did not reduce their blood-glucose level significantly. These findings probably suggest that the effective absorption of liposome-entrapped protein is possibly dependent on the diabetic state of the animals, or the amount of the intact insulin absorbed may not be sufficient to produce any significant change in the blood-glucose level in normal rats. These possibilities are at present under investigation.

Experiments are also in progress to obtain more satisfactory and reproducible entrapments of insulin. The effect of the low solubility of insulin on entrapment and our suspicion that not all the gamma (γ) counts are necessarily associated with intact insulin is under investigation. The effect of varying lipid com-

Table 2
Effects of oral and intraperitoneal administration of insulin on blood-glucose level in normal and diabetic rats

Route of administration	Blood glucose content % of initial	
	in normal	in diabetic
Orally	100.0	42.0
(Liposome-entrapped	93.6	46.1
insulin)	114.6	62.4
	97.4	47.9
Intraperitoneally	29.8	16.5
(Free insulin)	17.7	24.8
	32.2	20.5

12 IU of insulin was administered to normal and diabetic rats of body weight 150–170 g. For the oral dose insulin was given as liposome-entrapped insulin and for the intraperitoneal administration free insulin was used. The blood samples were taken before and 3 h after insulin administration. The results are expressed as the percentage of the initial blood-glucose level. Each result refers to a single rat.

position and net charge of the liposomes on their possible use in diabetic therapy is also being considered.

The results discussed here indicate that liposome-entrapped insulin, when administered orally, is able to reduce the blood-glucose level in diabetic rats, whereas the same amount of free insulin administered orally has no effect on blood-glucose level. The effect of liposome-entrapped insulin on blood-glucose level when administered orally is comparable with that produced when it is administered intraperitoneally. Our results indicate that by encapsulating insulin within liposomes, it is protected from degradation by proteolytic digestion in the gastro-intestinal tract, and retains its hypoglycemic activity after being absorbed in the circulation. Thus liposomes open up a new scope for the oral therapeutic use of proteins and, probably, other substances which are otherwise not suitable for oral administration.

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