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Enhanced nasal absorption of insulin in rats using lysophosphatidylcholine

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Summary

The effectiveness of L-α-lysophosphatidylcholine (LPC) as an enhancer for the nasal delivery of large peptide drugs was investigated in rats using insulin as a model drug. Intranasal insulin (16.7 IU/kg) solutions in combination with 0.5% of this enhancer produced a 65% decrease in blood glucose levels, which was similar to the decrease obtained using laureth-9 as a known enhancer. The two main constituents of LPC, namely, the palmitoyl component (72%) and the stearoyl component (24%) produced similar effects, at a concentration of 0.5%, to that of LPC, thus indicating that both of these lysophospholipids are equally potent absorption enhancers with potential in nasal delivery.

Introduction

In the last few years the nasal route of delivery has been established as a potential alternative way of administering novel bio-genetically engineered drugs including well known substances such as insulin. For decades and also presently insulin has been administered by injection to the patient, usually subcutaneously but also intramuscularly. For both type I and type II diabetic patients, parenteral insulin therapy means local discomfort and perceived disruption of normal life-style. Many type II diabetic patients entirely refuse such therapy due to their unwillingness to accept the associated physical and social trauma (Moses et al., 1983). Consequently, much effort has been dedicated to the formulation of alternative effective delivery systems other than the parenteral. Thus, insulin has been administered orally (Shichiri et al., 1978; Damge et al., 1988), ocularly (Lee et al., 1988), vaginally (Morimoto et al., 1982), rectally (Ichikawa et al., 1980; Ritschel et al., 1987), transdermally (Siddiqui et al., 1987) and pulmonarily (Jones et al., 1988). However, the success, in terms of reproducibility and bioavailability, has been very limited.

The nasal route has also been employed as a non-parenteral route for the delivery of insulin. Peptides and proteins (including insulin) are not normally well absorbed from the nasal cavity – the bioavailability of simple aqueous solutions administered nasally being of the order of less than 5% relative to subcutaneous administration.

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In order to improve the bioavailability of such peptides, various groups have explored the possibility of enhancing the absorption across the epithelial membrane by means of so-called absorption enhancers. These enhancers have included nonionic surfactants, chelators, fatty acids and bile salts and similar molecules to include derivatives of fusidic acid. Generally, enhancers are believed to work by one or more of the following mechanisms: increasing membrane fluidity, opening up tight junctions, inhibiting the proteolytic enzymes that degrade peptides and proteins, reducing the viscosity of the nasal mucus. It has been shown that most of these absorption enhancers, especially bile salts and non-ionic surfactants, have an impact on the nasal membrane by disturbing its integrity (Hirai et al., 1981). Thus, in chronic use there is the possibility that the membrane could be permanently damaged. Hirai et al. (1981) found that insulin administered to rats in combination with sodium glycocholate or laureth-9 produced significant decreases (up to 60% of normal values) in blood glucose levels. Using an insulin–deoxycholate combination, Moses et al. (1983) found a bioavailability of 10% as compared to i.v. injection in normal and diabetic subjects. Similar results were reported by Saltzman et al. (1985) in type I diabetic patients. In a study in sheep, the use of sodium taurodihydrofusidate as an enhancer system for the nasal absorption of insulin resulted in a bioavailability of 16.4% relative to i.v. administration (Longenecker et al., 1987). This enhancer system is claimed to cause no local or systemic toxicity in rats or dogs in concentrations as high as 10% (Eppstein and Longenecker, 1988).

The aim of the present study was to evaluate the effectiveness of a lysophospholipid, namely, lysophosphatidylcholine (LPC) as an enhancer for nasal delivery of large peptide drugs. Lysophospholipids are surface-active amphiphilic compounds generated naturally in biological membranes by action of phospholipases. They are active in low concentrations and are converted within the membrane to normal cell components (Stafford and Dennis, 1988). LPC has earlier been shown by Illum et al. (1988) to considerably enhance the absorption of gentamicin in both rat and sheep animal models.

Insulin was chosen for the absorption studies since this drug is a good candidate for nasal delivery, has a suitable large molecular weight and the effect of the drug on the blood glucose level is readily detectable. The studies were performed in an in vivo rat model.

Materials and Methods

Materials

Semisynthetic human insulin, sodium salt (SHI, batch No. P371) was obtained from Novo-Nordisk, Denmark. L-α-Lysophosphatidylcholine (lysolecithin) (LPC), L-α-lysopl.osphatidylcholine, palmitoyl (LPCP), L-α-lysophosphatidylcholine, stearoyl (LPCS) and polyoxyethylene 9 lauryl ether (laureth-9) were purchased from Sigma Chemical Company Ltd. (Dorset, U.K.). All other chemicals were of reagent grade. The major constituents of LPC were reported by the Technical Services Department of the Sigma Chemical Company to be 71.7% of the palmitic type and 24.1% of the stearic type.

Stock solutions

Insulin stock solutions of 167 IU/ml were prepared in phosphate buffer of pH 7.3 each morning prior to the study. When required, each absorption enhancer was added to the insulin solution at a concentration of 0.5% (w/v).

Animal experiments

The rat in vivo experimental model described by Hirai et al. (1981) and modified by Fisher et al. (1987) was used to study the effect of lysolecithins on the intranasal (i.n.) absorption of insulin in solution. Non-diabetic male Wistar rats (JABU, Sutton Bonington, U.K.) of about 200 g were fasted overnight for about 20 h prior to the study. They were anaesthetized by i.p. injection of 60 mg/kg of pentobarbitone (60 mg/ml, Sagatal, May and Baker). The rats were tracheotomized, the oesophagus sealed and the carotid artery cannulated. 20 μl of the insulin solution containing 167 IU/ml of the drug with and without enhancer was instilled into the nasal cavity, using a Hamilton microsyringe. The dose of insulin received by

each rat was 16.7 IU/kg. For the subcutaneous (s.c.) route, insulin was administered in a volume of 100 μl to the rats at a dose of 1.33 IU/kg from a phosphate-buffered solution.

Blood samples (150 μl) were collected in fluoride oxalate blood tubes from the carotid artery at 10, 6 and 2 min prior to drug administration and at 5, 10, 15, 20, 40, 60, 120, 180, 240 and 300 min post-administration. The samples were kept on crushed ice and assayed for glucose content on the day of the study. The glucose level was determined by the glucose oxidase method on a Yellow Springs 23 AM glucose analyser.

Results and Discussion

The blood glucose levels of rats that received insulin intranasally, with or without enhancer, and subcutaneously are shown in Fig. 1 and in Table 1. The intranasal administration of 16.7 IU/kg insulin in phosphate buffer did not cause a decrease in blood glucose level but rather an increase from 4 to 5.5 mmol/l presumably caused by stress induced by operation and anaesthesia. This trend was also seen for the blood glucose levels following the intranasal administration of the control phosphate buffer. When the rats were given insulin intranasally in combination with 0.5% L-α-lysophosphatidylcholine the blood glucose level decreased from about 4.8 to 1.7 mmol/l; a decrease of the order of 65%. This decrease was very similar to the decrease in blood glucose level obtained following the administration of insulin intranasally in combination with 0.5% laureth-9. The combination of insulin with LPC, as an enhancer, provided a blood level–time profile very similar in shape to the one obtained for the subcutaneous injection. However, the dose given subcutaneously was 1.33 IU/kg compared to 16.7 IU/kg for the intranasal route.

The L-α-lysophosphatidylcholine used as an enhancer in these experiments and earlier experiments with gentamicin (Illum et al., 1988) is a mixture of two main constituents namely L-α-lysophosphatidylcholine, palmitoyl (71.7%) and L-α-lysophosphatidylcholine, stearoyl (24.1%). The individual absorption promoting effects of these two lysophospholipids are shown in Fig. 2 and Table 1.

It can be seen that the profiles of blood glucose levels after administration of insulin with either 0.5% LPCP or 0.5% LPCS are similar in shape and magnitude to that obtained following the intranasal administration of insulin with 0.5% LPC.

TABLE 1

Blood glucose levels (mmol/l) following i.n. (16.7 IU/kg) and s.c. (1.33 IU/kg) administration of sodium insulin (SHI) to rats. Values in brackets indicate S.E.M. values

Time (min)	Blank buffer	i.n. SHI	i.n. SHI +LPC	i.n. SHI +LPCP	i.n. SHI +LPCS	i.n. SHI +L9	s.c. SHI
−10	4.2 (0.21)	3.9 (0.03)	4.8 (0.21)	4.9 (0.16)	3.5 (0.30)	3.9 (0.19)	3.7 (0.00)
−6	4.0 (0.24)	4.0 (0.15)	4.8 (0.12)	4.9 (0.23)	3.7 (0.27)	4.0 (0.19)	3.6 (0.12)
−2	4.2 (0.27)	4.0 (0.12)	4.8 (0.11)	4.8 (0.15)	3.9 (0.23)	4.0 (0.20)	3.6 (0.06)
5	4.1 (0.19)	3.9 (0.00)	5.0 (0.09)	4.8 (0.16)	4.0 (0.10)	4.0 (0.19)	3.6 (0.13)
10	4.2 (0.20)	4.2 (0.23)	4.1 (0.04)	4.2 (0.09)	3.5 (0.14)	3.5 (0.17)	3.4 (0.06)
15	4.3 (0.27)	3.9 (0.18)	3.8 (0.12)	3.6 (0.19)	3.2 (0.19)	3.0 (0.13)	2.8 (0.15)
20	4.4 (0.23)	4.1 (0.23)	3.7 (0.05)	3.4 (0.22)	2.8 (0.21)	2.8 (0.17)	2.5 (0.09)
40	4.4 (0.28)	4.3 (0.15)	2.7 (0.21)	2.1 (0.32)	2.1 (0.19)	1.9 (0.21)	1.6 (0.18)
60	4.5 (0.23)	4.6 (0.07)	2.0 (0.15)	1.7 (0.27)	1.6 (0.07)	1.7 (0.05)	1.1 (0.09)
90	4.8 (0.31)	4.7 (0.03)	1.8 (0.10)	1.7 (0.22)	1.5 (0.09)	1.8 (0.12)	1.3 (0.09)
120	4.9 (0.33)	5.1 (0.01)	1.7 (0.13)	1.6 (0.25)	1.5 (0.11)	1.9 (0.24)	1.6 (0.23)
180	5.0 (0.50)	4.8 (0.09)	1.7 (0.17)	2.2 (0.66)	1.4 (0.12)	2.4 (0.54)	2.4 (0.46)
240	5.6 (0.32)	5.0 (0.19)	2.6 (0.23)	3.5 (1.27)	1.9 (0.21)	2.8 (0.76)	3.2 (0.38)
300	5.6 (0.45)	5.4 (0.25)	3.9 (0.54)	4.4 (1.27)	3.0 (0.12)	3.6 (0.89)	4.1 (0.32)

L9: laureth-9; other abbreviations as in text.

Thus, the present results indicate that the lysophospholipids are very potent nasal absorption enhancers giving effects not dissimilar to the effect obtained with the non-ionic surfactant enhancer laureth-9. In a study by Hirai et al. (1981), non-ionic ether type surfactants, such as laureth-9, were found to have high haemolytic activities and a marked effect on the nasal mucosa. The alteration of the nasal mucosa after administration of insulin with laureth-9 was significant and complete restoration was not observed within 24 h. Thus, such an enhancer will not be suitable for drugs used in chronic treatment. The effect of LPC or other lysophospholipids on the nasal membrane has not been reported in the literature.

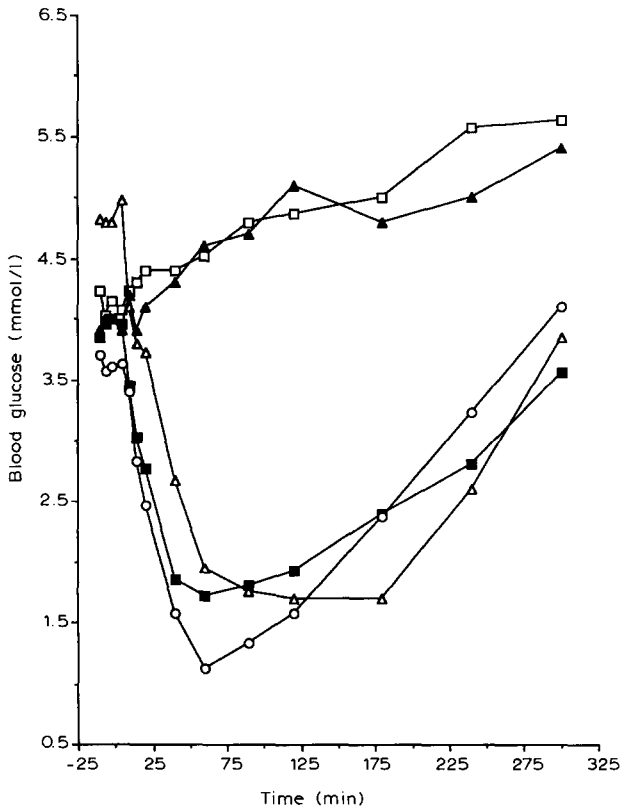

Fig. 1. Blood glucose levels following i.n. (16.7 IU/kg) and s.c. (1.33 IU/kg) administration of sodium insulin (SHI) to rats. (□) i.n. blank phosphate buffer solution as control ($n = 4$); (▲) i.n. SHI solution ($n = 3$); (■) i.n. SHI solution containing 0.5% laureth-9 ($n = 4$); (△) i.n. SHI solution containing 0.5% L-α-lysophosphatidylcholine ($n = 4$); (○) s.c. SHI solution ($n = 3$).

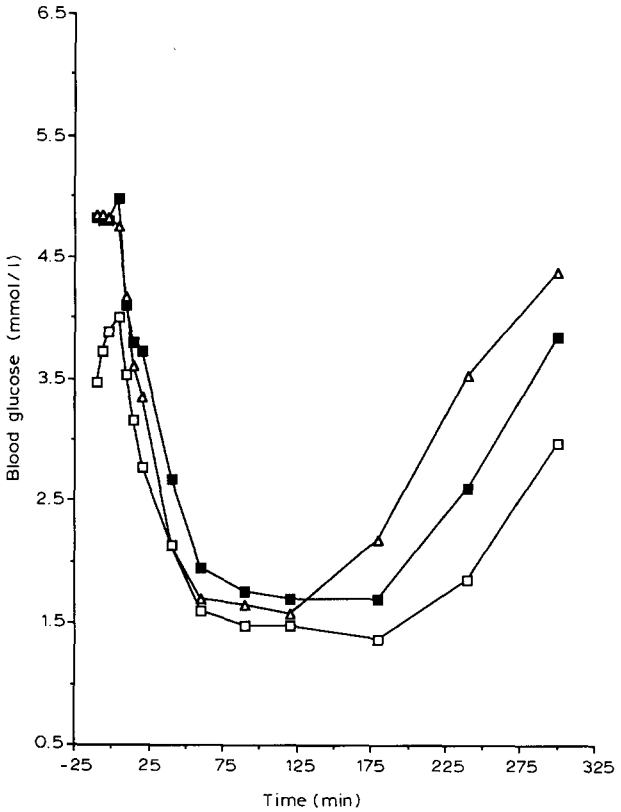

Fig. 2. Blood glucose levels following i.n. administration of sodium insulin (SHI) in combination with lysophosphatidylcholines to rats ($n = 4$). (■) SHI solution containing 0.5% L-α-lysophosphatidylcholine; (△) SHI solution containing 0.5% L-α-lysophosphatidylcholine, palmitoyl; (□) SHI solution containing 0.5% L-α-lysophosphatidylcholine, stearoyl.

We are currently evaluating this effect. However, previous studies have evaluated the effect of LPC on the gastric mucosa (Maksem et al., 1984), the intestinal mucosa (Talbot et al., 1984; Tagesson et al., 1985; Bolin et al., 1986) and the oesophageal mucosa (Salo et al., 1987). Generally, LPC causes damage to the gastric mucosa. However, this may mainly be due to the synergistic effect seen between HCl and LPC as shown by Salo et al. (1987) on oesophageal mucosa. In this study LPC administered in aqueous solutions at 0.2%, was found to cause no morphological damage to the mucosa when perfused without HCl into isolated segments of rabbit oesophagus. LPC has also been found to rapidly induce mucosal penetration of horseradish peroxidase in the intestines of guinea pigs without

any ultrastructural evidence of mucosal damage (Talbot et al., 1984). Similarly, Tagesson et al. (1985) found that 20 mmol LPC increased the permeability of rat ileum to macromolecules. However, at high concentrations, the authors also observed a damaging effect on the membrane. In the same way, LPC in a concentration of 1% was found to change morphologically the epithelium of the intestinal walls of rats (Bolin et al., 1986).

Thus it seems that, at low concentrations of LPC, the morphological changes induced by LPC on membranes are minimal although the enhancer at such concentrations significantly enhances the mucosal penetration of even large molecules. These observations are consistent with the fact that concentrations of LPC, too low to cause lysis of cells, still induce structural changes in membranes and affect enzyme activity (Weltzien, 1979). It should also be noted that fat emulsions given daily in high doses to patients are stabilised with phospholipids (1.2%) of which 4% is normally LPC giving an overall concentration of LPC of 0.05% (Grit et al., 1989; Illum et al., 1989).

It can be concluded that LPC appears to be a potentially effective absorption enhancer for nasal delivery of peptide and protein drugs. In animal models where the mucociliary function is intact, this effect is enhanced considerably when used in combination with a starch microsphere delivery system (Farraj et al., 1989).

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