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Effect of addition of hyaluronic acid to highly concentrated insulin on absorption from the conjunctiva in conscious diabetic dogs

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Abstract—Insulin absorption from the conjunctiva was investigated in five pancreatectomized diabetic mongrel dogs, with the nasolacrimal ducts occluded by micro plugs to prevent insulin absorption from nasolacrimal membrane. In the anaesthetized state, highly concentrated porcine insulin (1000 units mL^{-1}, pH 7·4) was absorbed rapidly and significantly from the conjunctiva. Plasma immunoreactive insulin concentrations increased significantly up to 3 h after insulin administration to conjunctival membranes. Plasma glucose concentration decreased significantly compared with saline control experiments after insulin administration (10 units kg^{-1}) at 3 h (8·3 ± 0·1 vs 15·6 ± 0·6 mmol, $P < 0·01$). However, in the conscious state, there was no significant increase in the plasma insulin levels after topical insulin administration. To improve insulin absorption in the conscious state, we examined the effect of increasing viscosity of insulin preparation with hyaluronic acid. In anaesthetized experiments, there were no significant changes in the bioavailability of insulin after addition of hyaluronate (0·84 ± 0·11 vs 0·87 ± 0·05%). In the conscious state, with addition of hyaluronic acid, the area under the curve of plasma insulin concentration was significantly increased (1842 ± 383 vs 75 ± 24 m units min L^{-1}, $P < 0·01$). The bioavailability of insulin absorption was significantly increased after addition of hyaluronate (0·68 ± 0·14 vs 0·03 ± 0·01%, $P < 0·01$). From this study we could demonstrate that the conjunctiva is a potential route for insulin administration, and increased viscosity by the addition of hyaluronate was found effective in increasing the bioavailability of insulin absorption from conjunctival membrane in the conscious state.

Since its discovery in 1921, insulin has usually been administered by subcutaneous injection to treat insulin-dependent diabetic (IDDM) patients. Although the subcutaneous multiple-injection therapy is effective in controlling blood glucose concentrations and diabetic complications such as retinopathy in IDDM patients, it would be more convenient if the insulin could be administered by other non-invasive methods. Various sites have been examined for this purpose; including intestine (Moses & Flier 1987), rectum (Kawamori & Shichiri 1982; Yagi et al 1983), nasal passages (Hirai et al 1978; Frauman et al 1987), lung (Wigley et al 1971; Kohlert et al 1984; Salzman et al 1985) and buccal membrane (Nagai 1985).

Recently, we have found that insulin is absorbed rapidly and in significant quantities from the conjunctival membrane in anaesthetized normal and diabetic dogs (Nomura et al 1990). However, in order to assess the conjunctiva as a potential route for insulin administration to control blood glucose and to prevent retinopathy in diabetic patients, the dynamics of insulin absorption in the conscious state needs to be clarified in experimental animals.

In this study, insulin absorption from the conjunctival membrane was investigated in conscious pancreatectomized diabetic dogs. The effects on bioavailability by increasing the viscosity of the insulin preparation by the addition of hyaluronic acid were examined to test conjunctiva as a potential route for insulin administration.

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Materials and methods

Five mongrel dogs made diabetic by total pancreatectomy (age: 4 ± 1 years, body weights 11 ± 1 kg, mean ± s.e.m.) were used in this study. After complete recovery from the surgery, experiments were carried out using a cross-over design in conscious or anaesthetized dogs within three weeks; there were no significant differences and changes in body weights of diabetic dogs between experiments. For frequent blood collections, an indwelling catheter was placed in the femoral vein. These diabetic dogs were maintained on porcine intermediate-acting insulin given by subcutaneous injection (0·5–1 units kg^{-1}) in the morning, before feeding, but this injection was withheld for 48 h on the day before and on the day of experiment; water was freely available throughout.

Highly concentrated insulin solutions with sodium hyaluronic acid or without sodium hyaluronic acid were prepared as follows. A total of 30 000 int. units (1·172 g) of porcine crystalline insulin (Novo-Nordisk, Denmark) was dissolved completely in 0·04 M HCl, and the pH was adjusted to 7·4 with 0·5 M NaOH. Distilled water was added to make final insulin concentrations of 1000 and 100 units mL^{-1}. To modify the viscosity of the insulin preparation, 0·36% sodium hyaluronic acid (Roman Kogyo Co. Ltd, Tokyo, Japan) was added. The viscosity of the insulin preparations were measured with a viscometer (Visconic, Tokyo, Japan), and the viscosity was found to be 4 ± 1 cPs without hyaluronic acid and 164 ± 5 cPs with hyaluronic acid ($n = 5, P < 0·01$).

Animal experiments were started at 0900 h after an overnight fast. In the anaesthetized experiments, diabetic dogs were anaesthetized in the morning with pentobarbitone before experiments. In conscious experiments, diabetic dogs were trained to stand quietly during the study. Insulin preparation was administered to the lower palpebral conjunctive as in the anaesthetized experiments. However, in both experiments, to prevent insulin absorption through the nasolacrimal membrane, nasolacrimal ducts were carefully occluded with Punctum Plug (Toray Seisaku Co. Ltd, Tokyo, Japan) on the day before experiments and confirmed to be in the correct position just before and after the experiments. Insulin was administered at dosages of 1 or 10 units kg^{-1} in a constant volume of 10 μL kg^{-1}. As a control, saline (10 μL kg^{-1}) was administered in the same manner to the lower ocular conjunctive. Blood samples were collected through an indwelling catheter at 0, 5, 10, 15, 20, 30, 60, 90, 120, and 180 min after topical insulin administration. Plasma glucose concentration was measured with a glucose analyser (Beckman Instruments, Fullerton, CA) using the glucose oxidase method. Plasma immunoreactive insulin concentration (IRI) was measured by radioimmunoassay.

The bioavailability of each insulin preparation was calculated using the following equation in comparison with 0·2 units kg^{-1} intravenous insulin in the same dogs:

$$\text{Bioavailability}\,(\%) = \frac{AUC_{IRI}}{AUC_{IRI(i.v.)}} \times \frac{0·2}{\text{Insulin dosage}} \times 100\,(\%) \quad (1)$$

where AUC_{IRI} is the area under the curve for 180 min after insulin administration to the conjunctival sac, $AUC_{IRI(i.v.)}$ is the

Table 1. Effects of insulin concentration on the insulin absorption through conjunctival membrane and glucose responses in anaesthetized and conscious diabetic dogs.

Time (min)	0	5	10	15	20	30	60	90	120	180
Anaesthetized dogs										
1 unit kg^{-1}										
Glucose (mmol)	16·7 ± 1·4	16·5 ± 1·4	16·4 ± 1·4	16·2 ± 1·4	15·9 ± 1·4	15·3 ± 1·5	14·1 ± 1·7	13·4 ± 1·7	13·1 ± 1·7	13·1 ± 1·6
IRI (μunits mL^{-1})	2 ± 1	6** ± 1	10** ± 1	11** ± 1	14** ± 1	17** ± 1	13** ± 2	11** ± 2	8** ± 1	4** ± 1
10 units kg^{-1}										
Glucose (mmol)	16·6 ± 0·6	16·2 ± 0·6	15·8 ± 0·5	15·7 ± 0·5	15·3* ± 0·5	14·1** ± 0·5	11·6** ± 0·3	10·5** ± 0·2	9·4** ± 0·2	8·3** ± 0·1
IRI (μunits mL^{-1})	2 ± 1	8** ± 1	16** ± 1	24** ± 1	30** ± 4	33** ± 3	20** ± 1	14** ± 1	10** ± 2	5** ± 1
Saline										
Glucose (mmol)	17·6 ± 0·5	17·6 ± 0·6	17·4 ± 0·6	17·4 ± 0·6	17·2 ± 0·6	16·9 ± 0·6	16·6 ± 0·6	16·2 ± 0·5	15·9* ± 0·6	15·6* ± 0·6
IRI (μunits mL^{-1})	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1
Conscious dogs										
10 units kg^{-1}										
Glucose (mmol)	17·1 ± 0·9	17·0 ± 0·9	17·1 ± 0·9	17·0 ± 0·9	16·9 ± 1·0	16·6 ± 1·0	16·4 ± 1·0	16·4 ± 1·0	16·3 ± 0·9	16·1 ± 0·7
IRI (μunits mL^{-1})	1 ± 1	2 ± 1	3 ± 1	3 ± 1	3 ± 4	2 ± 3	1 ± 1	1 ± 1	1 ± 2	1 ± 1
Saline										
Glucose (mmol)	17·6 ± 1·0	17·5 ± 1·0	17·4 ± 1·0	17·6 ± 1·0	17·4 ± 1·0	17·1 ± 1·0	16·9 ± 1·0	16·9 ± 1·0	16·6 ± 0·9	16·7 ± 0·9
IRI (μunits mL^{-1})	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1

*$P < 0.05$, **$P < 0.01$ compared with time 0. All data are presented as mean ± s.e.m. (n = 5).

Table 2. Changes in plasma IRI and glucose concentrations following topical application of highly concentrated insulin to the lower conjunctival sacs with or without sodium hyaluronic acid in anaesthetized diabetic dogs.

Time (min)	0	5	10	15	20	30	60	90	120	180
10 units kg^{-1} without hyaluronic acid										
Glucose (mmol)	16·4 ± 0·8	16·3 ± 0·8	16·0 ± 0·8	15·5 ± 0·8	15·2 ± 0·9	14·2 ± 1·0	12·9* ± 0·8	12·1* ± 0·8	11·3* ± 0·8	10·2* ± 0·7
IRI (μunits mL^{-1})	2 ± 1	10** ± 2	19** ± 3	21* ± 4	18* ± 2	30* ± 2	18* ± 2	14* ± 2	11* ± 3	10* ± 1
10 units kg^{-1} without hyaluronic acid										
Glucose (mmol)	16·6 ± 0·6	16·2 ± 0·6	15·8 ± 0·5	15·7 ± 0·5	15·3* ± 0·3	14·1** ± 0·5	11·6** ± 0·3	10·5** ± 0·2	9·4** ± 0·2	8·3** ± 0·1
IRI (μunits mL^{-1})	2 ± 1	8** ± 1	16** ± 1	24** ± 1	30** ± 4	33** ± 3	20** ± 1	14** ± 1	10** ± 2	5** ± 1
Saline										
Glucose (mmol)	17·6 ± 0·6	17·6 ± 0·6	17·4 ± 0·6	17·4 ± 0·6	17·2 ± 0·6	16·9 ± 0·6	16·6 ± 0·6	16·2 ± 0·5	15·9* ± 0·6	15·6* ± 0·6
IRI (μunits mL^{-1})	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1

*$P < 0.05$, **$P < 0.01$ compared with time 0.

area under the curve for 180 min after intravenous insulin injection (0·2 units kg^{-1}), and insulin dosage is the insulin dose administered to the ocular conjunctive (1 or 10 units kg^{-1}). All data are expressed as the mean ± s.e.m. and statistical analysis was carried out using the paired t-test.

Results

In the anaesthetized diabetic dogs after administration of 10 units kg^{-1} insulin preparations in the same volume, plasma IRI levels were increased (Table 1). Significant increases in plasma IRI levels persisted for 3 h. Plasma glucose concentrations were decreased significantly. Conscious diabetic dogs showed no significant increases in plasma IRI levels even at the dose of 10 units kg^{-1} (Table 1). The area under the plasma IRI concentration-time curve up to 180 min showed no significant difference compared with control saline administration.

The effect of increasing viscosity of the insulin preparation with hyaluronic acid on insulin absorption and plasma glucose was investigated in anaesthetized diabetic dogs (Table 2). The increment in plasma IRI concentrations after administration of insulin with hyaluronic acid was the same as insulin without hyaluronic acid. There were no significant differences in the area under the plasma IRI concentration curves during 3 h after insulin application (AUC_{IRI}) between the preparations. There were also no significant differences in the calculated bioavailability of the two insulin preparations (Table 3).

There was no significant increase in plasma IRI levels after conjunctival administration of 10 units kg^{-1} insulin (Table 4). However, following administration of the same dose with added hyaluronic acid, there were significant increases in plasma IRI concentrations and plasma glucose concentrations decreased significantly at 180 min compared with the fasting level.

Judged on the changes in AUC for IRI and glucose, the

Table 3. Bioavailability of insulin absorption following topical application of highly concentrated insulin to the lower conjunctival sacs with or without sodium hyaluronic acid in anaesthetized diabetic dogs.

Insulin dosage	$Area_{PG}$ (mmol min L^{-1})	AUC_{IRI} (m units min L^{-1})	Bioavailability (%)
10 units kg^{-1} without hyaluronic acid	992 ± 70**	2349 ± 128**	0·87 ± 0·05
10 units kg^{-1} with hyaluronic acid	710 ± 52**	2258 ± 305**	0·84 ± 0·11
Saline	236 ± 29	20 ± 5	—

**$P < 0.01$ compared with saline administration. $Area_{PG}$ is the area of plasma glucose concentration decreased from time 0. AUC_{IRI} is the area under the curve of IRI for 180 min. Bioavailability was calculated by comparison with 0·2 units kg^{-1} intravenous insulin administration.

Table 4. Changes in plasma IRI and glucose concentrations after conjunctival administration of highly concentrated insulin with or without sodium hyaluronic acid in conscious diabetic dogs.

Time (min)	0	5	10	15	20	30	60	90	120	180
10 units kg^{-1} with hyaluronic acid										
Glucose (mmol)	17·3 ± 0·9	17·2 ± 0·9	17·1 ± 0·9	16·8 ± 0·8	16·5 ± 0·8	16·0 ± 0·9	14·9 ± 0·9	14·4* ± 0·9	13·6* ± 0·8	12·8** ± 0·8
IRI (μunits mL^{-1})	2 ± 1	6 ± 2	11 ± 4	21* ± 6	21* ± 4	19* ± 7	16* ± 4	11* ± 3	10* ± 3	4 ± 1
10 units kg^{-1} without hyaluronic acid										
Glucose (mmol)	17·1 ± 0·9	17·0 ± 0·9	17·1 ± 0·9	17·0 ± 0·9	16·9 ± 1·0	16·6 ± 1·0	16·4 ± 1·0	16·4 ± 1·0	16·3 ± 0·9	16·1 ± 0·7
IRI (μunits mL^{-1})	1 ± 1	2 ± 1	3 ± 1	3 ± 1	3 ± 4	2 ± 3	1 ± 1	1 ± 1	1 ± 2	1 ± 1
Saline										
Glucose (mmol)	17·6 ± 0·6	17·6 ± 0·6	17·4 ± 0·6	17·6 ± 0·6	17·4 ± 1·0	17·1 ± 1·0	16·9 ± 1·0	16·9 ± 1·0	16·6 ± 0·9	16·7 ± 0·9
IRI (μunits mL^{-1})	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1

*$P < 0.05$, **$P < 0.01$ compared with time 0.

Table 5. Bioavailability of insulin absorption following conjunctival administration of highly concentrated insulin with or without sodium hyaluronic acid in conscious diabetic dogs.

Insulin dosage	$Area_{PG}$ (mmol min L^{-1})	AUC_{IRI} (m units min L^{-1})	Bioavailability (%)
10 units kg^{-1} without hyaluronic acid	193·1 ± 9·0	75 ± 24	0·03 ± 0·01
10 units kg^{-1} with hyaluronic acid	510·4 ± 48·9**	1842 ± 383**	0·68 ± 0·14**
Saline	134·0 ± 46·7	20 ± 5	—

*$P < 0.01$, **$P < 0.01$ compared with 10 units kg^{-1} without hyaluronic acid.

bioavailability of the insulin preparation was significantly increased with addition of hyaluronate (Table 5).

Discussion

Many sites have been studied as alternative routes for insulin administration to prevent diabetic complications (Moses & Flier 1987). In our previous study we found significant insulin absorption from conjunctival membrane using 500 units mL^{-1} insulin (Nomura et al 1990). As presented in this study, by increasing insulin concentrations from 100 to 1000 units mL^{-1}, we have confirmed that topically-applied insulin could be absorbed more quickly from the conjunctival membrane and a significant decrease in plasma glucose concentration could be obtained in anaesthetized diabetic dogs. However, simple administration of the highly concentrated insulin preparation to conjunctiva could not increase plasma IRI concentrations in the conscious state, probably because the insulin solution was rapidly washed away by palpebral movements and tears.

To overcome this situation, we have tried to investigate the effects of increasing viscosity of the insulin preparation. To increase viscosity, 0·36% (w/v) sodium hyaluronic acid, a substance naturally present in ocular vitreous fluid, was added; a 40-fold increase in viscosity was obtained. Drainage into the nasolacrimal cavity was prevented by occlusion of nasolacrimal ducts by Punctum Plug.

Before the study in conscious dogs, we examined the bioavailability of the insulin preparation with hyaluronate in anaesthetized dogs. The data in anaesthetized diabetic dogs clearly indicated that the addition of hyaluronic acid had no significant inhibitory effect on insulin absorption. In conscious experiments, there was a significant increase in plasma IRI levels and a significant decrease in plasma glucose concentrations after administration of the insulin with hyaluronic acid. As a result, bioavailability of insulin absorption was significantly increased.

These results suggest the conjunctiva as a possible convenient site for insulin administration, since the insulin absorption rate was rapid. However, in man the volume of the conjunctival sac is, at the most, 50 μL and it would be necessary to develop a more concentrated insulin preparation to reduce the volume of insulin drops (Nomura et al 1990). It would also be necessary to investigate the long-term effects of topical application of such insulin preparations.

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