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Insulin absorption from conjunctiva studied in normal and diabetic dogs

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Abstract—The dynamics of insulin absorption from the ocular conjunctiva of anaesthetized normal and pancreatectomized dogs have been examined. A porcine insulin preparation of 1000 units mL^{-1} (pH 8·0) was administered as either 1 or 10 units kg^{-1} to the upper conjunctival sacs of recumbent dogs following an overnight fast. Plasma insulin concentrations increased significantly at 5 min after the insulin administration. Plasma glucose concentrations decreased significantly, in both normal (given 10 units kg^{-1}) and diabetic dogs (given 1 unit kg^{-1} or 10 units kg^{-1}). There was a dose-dependent increase in plasma insulin concentration following conjunctival administration. Estimated absorption was significantly higher in diabetic than in normal dogs.

Various sites have been examined for possible insulin absorption (Moses & Flier 1987), including intestinal (Kawamori & Shichiri 1982), rectal (Kawamori & Shichiri 1982; Yagi et al 1983), nasal (Hirai et al 1978), pulmonary (Wigley et al 1971; Kohlert et al 1984), and buccal mucosal membranes (Nagai 1985). However, there have been no reports of insulin absorption from the ocular conjunctiva.

We have investigated insulin absorption from conjunctiva in anaesthetized normal and diabetic dogs and have tried to evaluate conjunctiva as a potential route for insulin administration to control blood glucose concentration.

Materials and methods

Animals. Five mongrel dogs (11 ± 2 kg, mean ± s.e.) were used as their own controls. As undosed controls, sterile 0·9% NaCl (saline) solution was applied to upper conjunctival sacs. Animals were then pancreatectomized.

Insulin preparations. 30 000 units (1·172 g) of porcine crystalline insulin (Sigma, St. Louis, MO) was dissolved in 22·9 mL of 0·04 M HCl, and the pH of the solution adjusted to 8·0 with 0·5 M NaOH: Distilled water was added to make up to 30 mL (1000 units mL^{-1}).

Methods. Following an overnight fast, dogs were anaesthetized with pentobarbitone, then laid in the recumbent position to minimize loss of insulin solution and its possible absorption through nasal membranes.

The insulin preparation, at either 1 unit kg^{-1} or 10 units kg^{-1}, was administered to the upper conjunctival sac in a random order. There was no leakage of insulin into the nasal cavity through naso-lacrimal ducts. All experiments were carried out in the morning.

Blood samples were collected through an indwelling catheter placed in the femoral vein. Plasma glucose concentrations (mmol L^{-1}) were measured by the Glucose Analyzer (Beckman Instruments, Fullerton, CA) using a glucose oxidase method. Plasma insulin concentrations (m units L^{-1}) were measured by radioimmunoassay, as immunoreactive insulin (IRI).

To evaluate the efficiencies of insulin absorption through conjunctiva, the area under the curve of insulin concentrations (AUC.IRI) was calculated and compared with that after i.m. insulin (0·2 units kg^{-1}) and bioavailability was determined according to the equation:

$$\text{Bioavailability} = \frac{(\text{AUC.IRI})}{(\text{AUC.IRI i.m.})} \times \frac{0{\cdot}2}{\text{Dose}} \times 100\%.$$

All the data were expressed as mean ± s.e. and statistical analysis was carried out by a paired *t*-test.

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Table 1. Changes in plasma glucose concentrations and plasma immunoreactive insulin concentrations (IRI) in normal dogs after conjunctival insulin administration at dosages of either 1 unit kg^{-1} or 10 units kg^{-1}.

Time (min)	−5	0	5	10	15	20	30	60	90	120	180
1000 units mL^{-1}: 1 unit kg^{-1} (n = 5)											
Plasma glucose concentration (mmol L^{-1}) ± s.e. ≤ 0·2	5·0	5·0	5·0	4·9	4·9	4·9	4·9	4·8	4·7	4·8	4·8
IRI (m units L^{-1}) ± s.e.m. = 1	8	8	9	9	10	11	11	9	9	8	7
1000 units mL^{-1}: 10 units kg^{-1} (n = 5)											
Plasma glucose concentration (mmol L^{-1}) ± s.e. ≤ 0·4	4·9	4·9	4·9	4·7	4·6	4·5	4·2	3·6*	3·3**	3·3*	3·8*
IRI (m units L^{-1}) ± s.e.m. = 1	9	9	10	12*	14**	17**	21**	18**	15**	13**	9
Saline (n = 5)											
Plasma glucose concentration (mmol L^{-1}) ± s.e. ≤ 0·2	4·7	4·8	4·8	4·8	4·8	4·8	4·8	4·8	4·7	4·8	4·7
IRI (m units L^{-1}) ± s.e.m. = 1	8	8	8	8	9	9	9	8	8	8	9

* $P < 0.05$, ** $P < 0.01$ (compared with data at time 0).

Table 2. Changes in plasma glucose concentrations and plasma immunoreactive insulin concentrations (IRI) in pancreatectomized diabetic dogs after conjunctival insulin administration at dosages of either 1 unit kg^{-1} or 10 units kg^{-1}.

Time (min)	−5	0	5	10	15	20	30	60	90	120	180
1000 units mL^{-1}: 1 unit kg^{-1} (n = 5)											
Plasma glucose concentration (mmol L^{-1}) ± s.e.m. ≤ 1·7	16·7	16·7	16·6	16·4	16·2	15·9	15·3	14·1	13·4	13·1	13·1
IRI (m units L^{-1}) ± s.e.m. = 1	2	2	6**	10**	11**	14**	17**	13**	11**	8**	4**
1000 units mL^{-1}: 10 units kg^{-1} (n = 5)											
Plasma glucose concentration (mmol L^{-1}) ± s.e.m. ≤ 0·6	16·8	16·6	16·2	15·8	15·7	15·3*	14·1**	11·6**	10·5**	9·4**	8·3**
IRI (m units L^{-1}) ± s.e.m.	2	2	8**	16**	24**	30**	33**	20**	14**	10**	5**
	1	1	1	1	1	4	3	1	1	2	1
Saline (n = 5)											
Plasma glucose concentration (mmol L^{-1}) ± s.e.	17·6	17·7	17·6	17·4	17·4	17·2	16·9	16·6	16·2	15·9	15·6
	0·5	0·6	0·6	0·6	0·6	0·6	0·6	0·6	0·5	0·6	0·6
IRI (m units L^{-1}) ± s.e.m. = 1	1	1	1	1	1	1	1	2	1	1	1

* $P < 0.05$, ** $P < 0.01$ (compared with data at time 0).

Results

Insulin absorption from palpebral conjunctiva in normal dogs. After insulin administration to the upper conjunctival sac (10 units kg^{-1}), plasma IRI concentrations increased significantly compared with fasting values, while plasma glucose concentrations decreased significantly from the fasting levels (Table 1).

Calculated areas of the plasma IRI concentrations compared with fasting values were significantly larger at the 10 units kg^{-1} dose compared with control experiments with saline. The area of the plasma glucose (PL.GL) concentrations compared with fasting plasma glucose values was also significantly larger (Table 3). However, we could not confirm any significant increases in either AUC.IRI or AUC.PL.GL at 1 unit kg^{-1} (Table 3). Calculated mean bioavailabilities of insulin absorption from conjunctival membrane in normal dogs were 0·5 ± 0·2% (1 unit kg^{-1}) and 0·3 ± 0·1% (10 units kg^{-1}).

Insulin absorption from conjunctiva in diabetic dogs. Before insulin administration, there were no significant differences in the mean fasting plasma glucose concentrations, as shown in

Table 3. Effects of conjunctival insulin administration on the area under the curve of plasma glucose (PL.GL) concentration and immunoreactive insulin concentration (IRI) over 3 h in anaesthetized normal and pancreatectomized dogs.

Dose	AUC.PL.GL	AUC.IRI
Normal dogs (n = 5)		
1000 units mL^{-1}		
1 unit kg^{-1}	32·8 ± 16·9	163 ± 64
10 units kg^{-1}	215·4 ± 25·5*	967 ± 106*
Saline	17·0 ± 8·7	32 ± 15
Pancreatectomized diabetic dogs (n = 5)		
1000 units mL^{-1}		
1 unit kg^{-1}	494·7 ± 42·4*	1510 ± 165*
10 units kg^{-1}	991·5 ± 156·7*	2349 ± 286*
Saline	236·1 ± 29·4	20 ± 5

AUC.PL.PG; Area under the curve of plasma glucose concentrations (mmol min L^{-1}).
AUC.IRI; Area under the curve of plasma IRI (m units min L^{-1}).
Data are presented as mean ± s.e.
* $P < 0.01$ (compared with saline).

Table 4. Bioavailability (%) of insulin preparations administered to upper conjunctival sacs in recumbent anaesthetized normal and diabetic dogs.

	Bioavailability (%)	
Dose	Diabetic	Normal
1000 units mL^{-1}		
1 unit kg^{-1}	6·6±0·7**	0·5±0·2
10 units kg^{-1}	1·0±0·1*	0·3±0·1

* $P<0·05$, ** $P<0·01$ (compared with normal).
Data are presented as means±s.e., n=5.

Table 2. Plasma IRI concentrations increased significantly as early as 5 min after insulin administration. Peak IRI concentrations and times to peaks were as follows: 17±1 m units at 30 min (1 unit kg^{-1}, $P<0·01$), and 33±3 m units L^{-1} at 30 min (10 units kg^{-1}, $P<0·01$) (Table 2). Plasma glucose concentrations decreased gradually, and the decrease was statistically significant after 3 h (Table 2).

Both AUC.IRI and AUC.PL.GL were significantly increased in diabetic animals when compared with control values (Table 3) and diabetic dogs absorbed more insulin from conjunctiva than did normal dogs (Table 4).

Discussion

Previously, we have reported the possible absorption of insulin from enteral and rectal membranes (Kawamori & Shichiri 1982; Yagi et al 1983). However, there were problems in the clinical usage of these routes; bioavailability was low (Moses & Flier 1987; Hirai et al 1978) and there was a wide variability of absorption (Hirai et al 1978), also, surfactants used to increase bioavailability from these routes had adverse effects on the membranes (Nagai 1985; Hirai et al 1981). Fusidic acid (Sliver et al 1985), bile acids (Nagai 1985; Hirai et al 1981; Sliver 1985), laureth-9 (Salzman et al 1985), and enamine (Kawamori & Shichuri 1982) were also reported to cause damage to membranes.

In the present study we have demonstrated that i) insulin absorption from conjunctiva was rapid; there were significant and constant increases in plasma IRI levels as early as 5 min after administration; ii) as a result, plasma glucose concentrations decreased significantly both in normal and diabetic animals; there seemed to be a dose-dependent increase in both peak plasma IRI and AUC.IRI after insulin administration to conjunctival sacs; iii) bioavailability of insulin was significantly higher in diabetic than in normal dogs; in diabetic animals absorption was between 1 and 7%, whereas in normal dogs it was less than 0·5%.

We have also investigated the day to day variations of both AUC.PL.GL and AUC.IRI in several dogs using the higher concentration insulin preparation. The coefficients of variation (CV) were: For AUC.PL.GL, 10·3% (1 unit kg^{-1}) and 15·4% (10 units kg^{-1}) and for AUC.IRI 17·9% (1 unit kg^{-1}) and 22·1% (10 units kg^{-1}).

For human application by this route chronic topical irritation or damage to ocular tissues (palpebral conjunctiva, sclera and cornea) might be of concern. We have already examined histological changes in rabbits by scanning electron microscopy; after two months of insulin administration, there were no significant changes in these tissues. However, further studies would be necessary before embarking on long-term clinical trials.

In this study we have demonstrated that insulin absorption through conjunctival membrane was quick and effective. For clinical application via this route, we need to clarify whether it is necessary to increase the bioavailability of insulin from conjunctiva. In man the volume of the conjunctival sac is at the most only 20–30 μL, so a higher insulin concentration would be needed. However, we found that the bioavailability of insulin was decreased when the insulin concentrations were increased. Thus, it will probably be necessary to increase bioavailability. We also need to take into consideration the effects of tear flow, which would clear the insulin solution soon after instillation in the conscious state. To prevent this, it might be useful to increase the viscosity of the insulin preparation. We have obtained good preliminary results by increasing viscosity. Finally, there is a need to increase stability of the insulin preparation itself as for clinical usage, pharmacological effects must be predictable and stable.

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