PHARMACEUTICAL STUDIES OF POLYACRYLIC ACID AQUEOUS GEL BASES: ABSORPTION OF INSULIN FROM POLYACRYLIC ACID AQUEOUS GEL BASES FOLLOWING RECTAL ADMINISTRATION IN ALLOXAN DIABETIC RATS AND RABBITS

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(Received May 9, 1979)

The effect of gel preparation on the rate of absorption of a rectally administered insulin which was suspended in a polyacrylic acid aqueous gel base (0.1%, pH 6.5) was investigated in the alloxan diabetic rats and rabbits. The preparation was administered into the *in situ* rectal loop in rats or infused directly into the rectum in rabbits, and the change in the blood glucose level and plasma insulin value was taken as a measure to evaluate the rate of rectal absorption of insulin. The blood glucose lowering effect in both rats and rabbits was dose-dependent, namely, a slight effect was observed at 1 IU/kg followed by a significant effect with 3 and 5 IU/kg, and a sharp hypoglycemic effect was recorded with 10 IU/kg which lasted for 5 hr. The plasma insulin level exhibited a rapid increase at 30 minutes after the dosing of the insulin gel at 3 IU/kg, which is indicative of a facilitated rectal rate of absorption of insulin by the polyacrylic acid aqueous gel base. However, the plasma level quickly diminished in 1 hr. Contrary to our expectation, an addition of a non-ionic surfactant such as Tween 80 under pH 4—8 did not show further enhancement of the absorption. In an attempt to simulate a clinical condition in which insulin was given after a meal, the insulin gel was administered into the rectal loop in diabetic rats following the *i.v.* injection of glucose. A satisfactory result was obtained in which 5 IU/kg dose was able to suppress a rise in the blood glucose after the glucose loading.

Keywords — insulin; rectal; polyacrylic acid; aqueous gel; blood glucose level; diabetic rats; diabetic rabbit; alloxan

Treatment of diabetes with injection of insulin constitutes a daily inconvenience for the patient. Furthermore, because of the problem of quadriceps muscle spasms due to parenteral injections of drugs, attempts are being made to develop a new nonparenteral method of administration in recent years. In particular, oral and rectal administration have been considered to be of value.

It is generally accepted that a proteinous hormone such as insulin is not effective by oral administration because it is degraded by proteolytic digestion and is hardly absorbed intact from the

digestive tract. However, numerous reports have appeared in recent years concerning the gastrointestinal absorption of insulin prepared in an oil based emulsion^{1,2)} or liposome base.^{3,4)} And in the case of rectal administration, insulin preparations on the bases of w/o/w type emulsion^{5,6)} have been reported to have a high therapeutic effect at low doses (1—5 IU/kg rabbits⁵⁾, dogs⁶⁾). But insulin preparations with the suppository bases in use today such as polyethylene glycol bases^{7,8)} and Witepsol⁷⁾ needed high doses (100 IU/kg rats, rabbits, dogs⁸⁾) for hypoglycemic effect.

Therefore, present study has been undertaken in an attempt to develop a polyacrylic acid aqueous gel suppository, which has a completely different properties from the conventional suppository bases.

Polyacrylic acid aqueous gels have been mainly used for cosmetics as a stabilizing agent of emulsion and adhesion of liquids, and for bases of ointment in case of medical preparations. However, there have been few reports concerning application to pharmaceutical preparation. The release of resorcinol from homogeneous ointment bases has been reported by Brunner and Speiser⁹⁾ and the pilocarpin aqueous gel ophthalmic preparation has been reported by Testard and Etter,¹⁰⁾ and Shoenwald *et al.*¹¹⁾ The high-viscosity vehicles made by using the aqueous gel can increase the bioavailability by increasing drug contact time with eyes, and these vehicles can prolong the effect of the drug as the result of the varied release rate in accordance with the change of viscosity.¹¹⁾

There is an advantage with polyacrylic acid aqueous gel bases in which the pH and viscosity can be adjusted over a wide range according to the type of drug to be used. In the case of a water-soluble drug, it is made possible to administer a drug in a form of solution to the rectum of animals or humans without creating a problem of leakage and a discomfort of having a bowel movement like that produced by PEG which tends to stimulate the secretion of water into the rectal lumen.¹²⁾

In this study, a rectal loop method of rats was carried out as a basic approach to the rectal administration and further a rectal administration to rabbits was carried out to evaluate its value for a clinical application.

MATERIALS AND METHODS

Materials—Polyacrylic acid (HIVISWAKO 105®) was obtained from Wako Pure Chemical Industries, Ltd., Japan, bovine insulin (24.9 IU/kg) from Sigma Co., Louis, Mo., alloxan monohydrate from Nakarai Chemical Co., Japan, and Tween 80 from Kishida Chemical Co., Japan.

Preparation of the Gel Bases—The gel was prepared by adding 10% NaOH solution to fit each pH into various concentrations of polyacrylic acid

presoaked in distilled water for 15 hours. The concentrations of the aqueous gel bases used in rectal administration were 0.1 and 1% (w/v) with the pH of 4.0, 5.0, 6.5, and 8.0. In the case of the base containing Tween 80, the concentration of Tween 80 was 5% (w/v) in the gel bases. Insulin was suspended in each gel base according to insulin doses.

Determination of Viscosity—The viscosity of the gel bases was measured with a cone plate viscometer (E type, Tokyo Keiki)¹³⁾ at 37°.

Animal Experiments—Wister strain male albino rats weighing 200–250 g and rabbits weighing 2–3 kg were used. Rats were made diabetic by intraperitoneal injection of a freshly prepared alloxan solution (200 mg/kg of body-weight),¹⁴⁾ and alloxan diabetic rabbits were prepared by injection of an alloxan solution into the ear vein (100 mg/kg).¹⁵⁾ They were checked to be diabetic by examining the blood glucose level within 7–10 days after alloxan administration. The blood glucose level in the diabetic animals was greater than 300 mg/dl of blood. In the following rabbit experiments, the concentration of blood glucose was checked once a week to confirm the diabetic condition. Normal and diabetic rats were fasted for 20 hr. Under pentobarbital anesthesia, the insulin gel was administered in the rectal loop (5 cm section above the anus) at the gel volume of 1 ml/200 g body weight. Rabbits were fasted for 20 hr and fixed on its back and the gel administered into the rectum at the volume of 0.5 ml/kg. Glucose tolerance test was performed with the alloxan diabetic rats being fasted for 20 hr. After a blood specimen was obtained at time 0, insulin gel preparation was administered into the rectal loop, and a glucose solution (100 mg/ml, 500 mg/kg) was injected into the femoral vein, immediately.

Blood Specimens and Determination of Blood Glucose and Plasma Insulin—Blood specimens were obtained from the femoral veins of rats and ear veins of rabbits at appropriate times. Blood glucose was determined according to the glucose-oxidase/peroxidase method¹⁶⁾ and plasma insulin was measured by the radioimmunoassay method¹⁷⁾ (Insulin Radioimmunoassay Kit, Daiichi Isotope,

Japan).

Morphological Studies on Rectal Tissue — An insulin gel preparation was administered into the rectal loop of a rat. Thirty minutes later, the rectum was isolated, rinsed with distilled water, fixed in 10% formalin and made into slices. They were observed under a light microscope being stained with hematoxylin-eosine.

RESULTS

Viscosity Characteristics of Polyacrylic Acid Aqueous Gel

The viscosity characteristics of the gel (HIVISWAKO 105®) measured with viscometer E type are shown in Fig. 1 and 2. Fig. 1 shows the relationship between viscosity and the rate of shear (sec^{-1}) at the gel concentration of 0.1, 0.5 and 1% (pH 6.5). It is classified as a non-Newtonian liquid as the viscosity became infinitely large near the 0 rate of shear. Fig. 2 shows the effect of pH on the viscosity of the gel. Quite a constant viscosity was observed over a wide range of pH 4.5—12 at both gel concentrations (0.1% and 1%).

Rectal Administration of Insulin

Table I indicates the blood glucose levels in both

alloxan diabetic rats and rabbits following the rectal administration of the insulin gel at two dose levels. In rats administered insulin at doses of 5 IU/kg and

FIG. 1. Viscosity Curves for Three Concentrations of Gel (HIVISWAKO 105®)
●, 0.1% Gel; ■, 0.5% Gel; ▲, 1.0% Gel. Viscosity was measured with cone plate viscometer at 37°. Each point represents the mean of three experiments.

TABLE I. Blood Glucose Levels Following Rectal Administration of Various Concentrations of Insulin Gel (pH 6.5) in Alloxan Diabetic Rats and Rabbits

Gel Conc.		0.1%		1%	
Insulin doses		5 IU/kg	10 IU/kg	5 IU/kg	10 IU/kg
Animal	Time after adm. (hr)	Blood glucose mg/dl			
Rats	0	138.1 ± 4.3	127.1 ± 8.5	134.3 ± 6.2	140.6 ± 4.2
	0.5	96.4 ± 4.1	86.5 ± 4.9	141.8 ± 8.2	147.3 ± 5.3
	1	96.9 ± 5.8	80.4 ± 4.1	108.4 ± 8.5	97.9 ± 8.9
	2	100.3 ± 6.7	91.7 ± 7.8	115.0 ± 10.3	103.7 ± 7.5
	3	115.0 ± 3.9	103.7 ± 6.5	126.8 ± 7.5	114.7 ± 7.9
	5	133.3 ± 5.7	105.1 ± 5.0	143.3 ± 8.2	121.8 ± 9.3
Rabbits	0	140.4 ± 16.8	142.6 ± 14.1	138.7 ± 6.3	142.0 ± 6.2
	0.5	114.7 ± 14.7	106.4 ± 9.1	145.9 ± 8.5	108.8 ± 6.2
	1	100.3 ± 9.2	89.2 ± 7.6	112.2 ± 7.5	101.0 ± 8.7
	2	112.2 ± 14.0	99.9 ± 6.3	115.8 ± 6.5	105.7 ± 9.4
	3	123.8 ± 11.7	99.2 ± 7.3	127.8 ± 9.1	110.1 ± 7.0
	5	136.7 ± 13.1	122.1 ± 8.5	132.6 ± 10.0	126.3 ± 9.0

Each value is a mean ± SEM of 5 animals.

10 IU/kg with 1% gel base, the blood glucose levels were not affected at the first 30 minutes. The

FIG. 2. Relationship between pH and the Viscosity of 0.1% and 1.0% Gel (HIVISWAKO 105®)

■, 0.1% Gel; ▲, 1.0% Gel.

Viscosity was measured at the rate of shear of 394 sec^{-1} with cone plate viscometer at 37° .

hypoglycemic effect was observed at 1 hr and continued for two hours. The blood glucose returned to the control level after 5 hours. On the other

FIG. 3. Change in Blood Glucose (—) and Plasma Insulin Levels (-----) Following Rectal Administration 0.1% Insulin Gel (pH 6.5) in Alloxan Diabetic Rats

●, 1 IU/kg; ■, 5 IU/kg; ▲, 10 IU/kg. Each point is a mean of 5 animals.

TABLE II. Dose Dependency of Blood Glucose and Plasma Insulin Levels Following Rectal Administration of 0.1% Insulin Gel (pH 6.5) in Alloxan Diabetic Rats

Insulin dose	1 IU/kg	3 IU/kg	5 IU/kg	10 IU/kg	30 IU/kg
(hr)	Blood glucose mg/dl				
0	127.8±9.8	122.0±6.9	138.1± 4.3	127.1± 8.5	144.7± 5.0
0.5	120.2±8.2	102.3±4.4	96.4± 4.1	86.5± 4.9	90.0± 5.7
1	125.4±7.8	100.0±4.6	96.9± 5.8	80.4± 4.1	77.8± 7.0
2	131.8±5.2	124.5±4.9	100.3± 6.7	91.7± 7.8	42.4± 7.1
3	136.1±6.6	132.4±7.7	115.0± 3.9	103.7± 6.5	51.3± 6.2
5	141.1±9.4	139.2±7.6	133.3± 5.7	105.1± 5.0	83.9±10.3
	Plasma insulin μU/ml				
0	7.2±1.6	6.1±0.8	6.5± 1.1	7.1± 1.3	5.8± 0.9
0.5	16.8±4.0	26.2±6.2	134.6±18.4	201.2±16.6	200.3±15.9
1	13.4±3.0	18.4±5.3	108.6±11.5	145.1±10.4	296.1±14.6
2	9.9±1.4	11.1±2.4	65.3± 6.1	121.7± 7.5	222.0±17.1
3	8.2±1.1	7.9±1.6	25.5± 4.6	74.6± 4.8	142.9± 8.0
5	6.9±1.2	5.0±1.8	9.2± 1.1	64.2± 4.9	116.0±11.2

Each value is a mean±SEM of 5 animals.

hand, when insulin (5 IU/kg and 10 IU/kg) was administered with the gel concentration of 0.1%, prominent hypoglycemic effects were observed at 30 minutes, reached a peak at 1 hr, and lasted well over 5 hours.

Tables II and III list the blood glucose levels and the plasma insulin concentrations following a rectal administration of various doses of the insulin gel (0.1%, pH 6.5) to alloxan diabetic rats and rabbits. Fig. 3 and 4 depicts the relation between the change in blood glucose levels and plasma insulin concentrations.

As shown in Table II and Fig. 3, no significant effect of insulin was observed at the dose of 1 IU/kg in the diabetic rats. At 5 IU/kg, a sharp drop in the blood glucose value (from 100 to 69.8%) was recorded at 30 minutes after administration which coincided with a sharp increase in the plasma insulin level (from 6.5 to 134.6 μ U/ml), indicating a rapid rate of absorption of insulin from the administered site. With the administration of 10 IU/kg and 30 IU/kg, even higher hypoglycemic effects were exhibited which lasted for 5 hr. Plasma insulin level reached a peak at 30 minutes or 1 hr, and rapidly declined thereafter.

Table III and Fig. 4 summarize the hypogly-

cemic effects following the rectal administration of the insulin gel to alloxan diabetic rabbits. As in the case of rats, a rapid hypoglycemic effect was noted in the rabbits. Blood glucose level decreased from

FIG. 4. Change in Blood Glucose (—) and Plasma Insulin Levels (---) Following Rectal Administration 0.1% Insulin Gel (pH 6.5) in Alloxan Diabetic Rabbits

●, 1 IU/kg; ■, 5 IU/kg; ▲, 10 IU/kg. Each point is a mean of 5 animals.

TABLE III. Dose Dependency of Blood Glucose and Plasma Insulin Levels Following Rectal Administration 0.1% Insulin Gel (pH 6.5) in Alloxan Diabetic Rabbits

Insulin dose	1 IU/kg	3 IU/kg	5 IU/kg	10 IU/kg
(hr)				
	Blood glucose mg/dl			
0	132.2±19.4	146.2±13.9	140.4±16.8	142.6±14.1
0.5	126.3±17.7	117.3±13.4	114.7±14.7	106.4± 9.1
1	127.9±16.2	108.9± 9.4	100.3± 9.2	89.2± 7.6
2	140.3±15.5	125.4±10.1	112.2±15.0	99.9± 6.2
3	145.1±14.1	136.8±10.4	123.8±11.7	99.2± 7.3
5	155.8± 9.5	155.9±12.1	136.7±13.1	122.1± 8.5
	Plasma Insulin μ U/ml			
0	5.8±1.2	6.6± 1.0	5.7± 1.0	6.6± 1.1
0.5	17.9±3.3	82.0±10.7	169.3±10.7	196.9±13.6
1	7.8±1.2	68.5± 8.9	143.7±12.2	160.9±14.1
2	6.9±1.0	44.9± 8.6	34.8±10.5	74.2± 6.8
3	6.7±1.3	29.4± 8.6	13.1± 4.0	41.9± 5.2
5	6.8±1.0	19.4± 4.2	12.2± 2.1	27.4± 2.6

Each value is a mean $\pm$ SEM of 5 animals.

146.2 to 108.9 mg/dl (from 100 to 74.5%) at 1 hr with a dose of 3 IU/kg, while plasma insulin level increased from 6.6 to 82.0 μ U/ml at 30 minutes. The effect continued into the 2nd and 3rd hr, and was still significant at 5 hr. The plasma insulin level reached its peak at 30 minutes at all dose levels, which suggests a rapid rate of absorption of insulin. The level declined quickly, thereafter.

The effect of pH of 0.1% gel on the absorption rate of insulin following rectal administration to diabetic rats is shown in Table IV. Although, the solubility of insulin into the gel seems to change by pH, which affects the rate of rectal absorption of insulin, no significant difference was observed in the blood glucose levels at various pH levels investigated.

The effect of gel containing 5% (w/v) Tween 80 on the absorption rate of insulin following rectal administration to the diabetic rats is shown in Table V. In this case, it was speculated that the dissolution rate of insulin in the gel and permeability of insulin to the rectal mucosa will increase by Tween 80, however, the effect was not significant. And though data are not shown, the viscosity of the gel was not affected in the case of preparations containing 5% (w/v) Tween 80.

Glucose Tolerance Test

The purpose of this test is to simulate the effect of rectally administered insulin gel on the blood glucose in patients after a meal. In this study, glucose solution was injected intravenously to the

FIG. 5. Blood Glucose Levels Following Rectal Administration of Insulin after Glucose Loading (500 mg/kg) in Alloxan Diabetic Rats

●, control; ▲, 5 IU/kg; ■, 10 IU/kg insulin; ▼, 30 IU/kg insulin. Gel concentration was 0.1% at pH 6.5. Each point is a mean $\pm$ SEM of 5 animals.

TABLE IV. Blood Glucose Levels Following Rectal Administration of Insulin Gel at Various pH in Alloxan Diabetic Rats

Gel pH	pH 4.0	pH 5.0	pH 6.5	pH 8.0
(hr)				
	Insulin dose 5 IU/kg			
	Blood glucose mg/dl			
0	138.5 $\pm$ 9.7	137.3 $\pm$ 14.8	138.1 $\pm$ 4.3	138.6 $\pm$ 8.2
0.5	92.0 $\pm$ 7.4	86.0 $\pm$ 6.9	96.4 $\pm$ 4.1	101.1 $\pm$ 12.4
1	92.1 $\pm$ 10.9	92.4 $\pm$ 9.7	96.9 $\pm$ 5.8	101.0 $\pm$ 13.3
2	97.8 $\pm$ 10.2	94.8 $\pm$ 9.2	100.3 $\pm$ 6.7	104.8 $\pm$ 14.5
3	129.7 $\pm$ 16.0	118.7 $\pm$ 10.6	115.0 $\pm$ 3.9	119.1 $\pm$ 13.0
5	144.9 $\pm$ 9.1	136.4 $\pm$ 9.2	133.3 $\pm$ 5.7	146.9 $\pm$ 8.8
	Insulin dose 10 IU/kg			
0	134.4 $\pm$ 13.2	137.4 $\pm$ 12.2	127.1 $\pm$ 8.5	128.6 $\pm$ 8.3
0.5	86.6 $\pm$ 9.0	83.7 $\pm$ 10.4	86.5 $\pm$ 4.9	69.5 $\pm$ 8.2
1	76.8 $\pm$ 5.8	75.8 $\pm$ 11.3	80.4 $\pm$ 4.1	75.3 $\pm$ 9.4
2	83.6 $\pm$ 7.1	83.4 $\pm$ 9.9	91.7 $\pm$ 7.8	81.1 $\pm$ 9.9
3	98.9 $\pm$ 11.5	103.3 $\pm$ 10.5	103.7 $\pm$ 6.5	91.5 $\pm$ 10.1
5	120.3 $\pm$ 10.5	114.4 $\pm$ 9.3	105.1 $\pm$ 5.0	100.7 $\pm$ 11.0

Each value is a mean $\pm$ SEM of 5 animals.

TABLE V. *Blood Glucose Levels Following Rectal Administration of 0.1% Insulin Gel (pH 6.5) Containing Tween 80 in Alloxan Diabetic Rats*

Tween 80 Conc.	0%	5%	0%	5%
Insulin dose	5 IU/kg	5 IU/kg	10 IU/kg	10 IU/kg
(hr)	Blood glucose mg/dl			
0	138.1±4.3	137.3±13.0	127.1±8.5	127.3±10.4
0.5	96.4±4.1	93.7±15.0	86.5±4.9	78.4± 7.8
1	96.9±5.8	82.9± 9.5	80.4±4.1	68.1± 9.6
2	100.3±6.7	94.6± 6.2	91.7±7.8	84.7± 9.0
3	115.0±3.9	106.1±11.0	103.7±6.5	97.5± 7.8
5	133.3±5.7	140.0±12.9	105.1±5.0	114.0± 9.5

Each value is a mean±SEM of 5 animals.

diabetic rats and the results of hypoglycemic effects were illustrated in Fig. 5. In the control group of diabetic rats without insulin gel, the blood glucose level rose to 270 mg/dl after the glucose loading, and this hyperglycemic condition persisted for 5 hr. The hyperglycemia was well suppressed in the insulin dosed group of animals (5 IU/kg). With 30 IU/kg dose, a hypoglycemic effect was noted at 1 hr and the effect lasted for 5 hr.

DISCUSSION

It has been generally considered that insulin can hardly be absorbed from intestines. However, this problem was overcome by using a gel preparation. A rapid rise in plasma insulin level with an accompanying fall in the blood glucose within 30

minutes was observed following the administration of an insulin gel preparation at the doses of 3 IU/kg and 5 IU/kg into the rat rectal loop or rabbit rectum. Particularly, a persistent hypoglycemic effect was demonstrated at the doses of 10 and 30 IU/kg. Its therapeutic effect was analogous to the rectal administration of insulin with w/o/w type emulsion.^{5,6)} Therefore, insulin rectal administration is more effective than oral administration, which required a high dose (80 IU/kg by Patel *et al.*³⁾ and 25 IU/kg by Shichiri *et al.*¹⁸⁾ to produce a hypoglycemic effect. The excellent rate of absorption of insulin in this study is considered to be due to the accelerating effect of polyacrylic acid aqueous gel on rectal absorption. As illustrated in the photograph of Fig. 6, no mucosal damage by the gel was observed and the gel preparation was absorbed on to the surface of the rectal mucosa like a film. In addition, a water-soluble high molecular compound such as polyacrylic acid is capable of lowering the surface tension of the mucosal surface to increase the wetting area of contact.¹⁹⁾ Therefore, it can be considered that polyacrylic acid aqueous gel increased the absorption capacity of the rectum by the above mechanism.

A possible explanation for the higher rate of insulin absorption from 0.1% gel base than from 1% preparation (Table I) may be that the release rate of insulin from the gel film on the surface of the mucosa is higher at lower gel concentrations. This

FIG. 6. *Histological Observation of the Rectal Mucosa One Hour after the Rectal Administration of Insulin Gel*

assumption is supported by the report of Brunner and Speiser⁹⁾ on the release rate study of resorcinol from an aqueous gel in which a higher release rate was obtained with the gel of lower viscosity. According to the report of Hirai,²⁰⁾ the rate of absorption of insulin from the nasal mucosa of dogs was most efficient at pH 3, however, pH did not affect the rate of rectal absorption of insulin from insulin gel in our experiment (Table IV).

Thus, it is considered that the higher diffusion rate of insulin in gel of lower viscosity brings about a rapid rate of absorption of insulin, and that its absorption rate is controlled by the viscosity of the gel because the gel viscosity was constant at pH 4.5—12 (Fig. 2).

Based on the results obtained in the glucose tolerance test, in which the rectally administered insulin gel at the insulin dose of 5 IU/kg was able to suppress a rise in the blood glucose level following a glucose loading, and a considerable lowering in the blood glucose was noted with 10 and 30 IU/kg (Fig. 5), it is suggested that this insulin gel given before meal to patients may be able to suppress a rise in blood glucose level.

Concerning the stability of the insulin gel preparation, it was stored for a month at 5° and subjected to a potency test. The blood glucose lowering effect was the same as a fresh preparation. From the facts described above, we may conclude that the polyacrylic acid aqueous gel base possesses a highly facilitating effect on the absorption rate of insulin from the rectum. This suggests that the insulin gel preparation by rectal administration may have a potential of being applied to clinical practice.

Acknowledgement The authors are grateful to Tōko Yakuhin Industry Co., Ltd. for the preparations of gel.

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