

Increase of blood glucose concentrations in diabetic patients with glucagon drops¹

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TRACT Hypoglycemic crisis is a common occurrence in diabetic patients. In order to reverse the hypoglycemia, glucagon eyedrops at concentrations of 2.5%, 5.0%, and 7.5% were instilled to the eyes of diabetic patients who were fasted overnight. The glucagon eyedrops raised the blood glucose efficiently in a dose-dependent manner and peaked at 30 min after drug instillation. At 2.5%, glucagon raised the blood glucose 0.83 mmol · L⁻¹ which exceeded the minimal requirement of 0.56 mmol · L⁻¹ increase in blood glucose level at hypoglycemic crisis. At 5.0% and 7.5%, glucagon eyedrops increased the blood glucose level further to 1.76 and 1.91 mmol · L⁻¹, respectively. These results indicate that glucagon can be delivered effectively through ocular route to raise systemic blood glucose for the treatment of hypoglycemic crisis.

WORDS glucagon; ophthalmic solutions; insulin-dependent diabetes mellitus; blood glucose; hypoglycemia

Hypoglycemic crisis is a frequent occurrence to diabetic patients who are either overdosed with hypoglycemic agents or due to delay of the meal time and/or not exercised. When the crisis occurs at home outside the hospital and without medical attendants, patients are often unable to take care of themselves with glucagon injection and/or glucose intake. As a result, it leads to irreversible coma and/or death. It has been reported that 0.2% glucagon eyedrops instilled to the rabbit eye is able to raise

rabbits blood glucose concentration from 5.6 mmol · L⁻¹ (100 mg%) to 13.4 mmol · L⁻¹ in 30 min⁽¹⁾. Since the body weight of human is approximately 20 times heavier than that of rabbits, at least 20 times larger amount of glucagon has to be instilled into eyes. In order to test the hypothesis that the blood glucose of human patients can be raised effectively within 30 min after instillation of glucagon eyedrops, various concentrations of glucagon solutions were instilled to human patients and their effects on blood glucose concentrations determined. It was exciting to observe that the human blood glucose concentrations can be raised effectively by glucagon eyedrops⁽²⁾.

MATERIALS AND METHODS

Subjects Forty-three patients with non-insulin-dependent diabetes mellitus were recruited in this study with 12 males and 31 females. Their ages ranged from 38 to 76 a with the duration of diabetes mellitus ranging from 1 through 30 a. The body weight ranged from 44 to 81 kg with an average of 62.3 kg (Tab 1). No hepatic malfunction was noted in routine blood chemistry screening.

Materials and methods Glucagon purchased from Novo Industri A/S (Copenhagen, Denmark) was freshly dissolved and adjusted to pH 9.5. This solution was centrifugated through Centricon 3 according to the manufacturer (Amicon, MA) and adjusted with phosphate buffer saline (PBS) to make the different concentrations of glucagon solution at 2.5, 5, and 7.5%. All the procedures were conducted in an aseptic

Tab 1. Diabetic patients instilled with glucagon (phosphate buffer saline as control).

Eyedrop	Patient	Sex	Age /a	Body height /cm	Body weight /kg	Duration of diabetes /a	Insulin medica- tion	Oral hypo- glycemic agents	Diet control	Blood glucose /mmol L ⁻¹	
Phosphate buffer saline	1	F	57	156	61	6		+		4.4	
	2	F	42	152	60	13	+			17.2	
	3	F	38	154	60	1			+	7.2	
	4	F	63	153	59	15		+		6.4	
	5	F	54	146	44	12	+			10.6	
	6	M	48	165	72	1			+	7.4	
	7	F	56	156	63	16	+		+	7.0	
	8	F	66	157	50	17	+			9.6	
	9	M	71	171	65	13	+			14.4	
	10	F	54	157	56	9	+			8.2	
	11	F	57	148	64	1			+	6.3	
	12	M	61	170	75	5			+	11.6	
	13	M	72	165	65	16			+	6.9	
	14	F	56	160	80	0.5				+	5.0
	15	M	76	162	60	13	+			7.6	
	$\bar{x} \pm s$		58 ± 11	158 ± 7	62 ± 9	9.2 ± 6.2				8.66 ± 3.9	
Glucagon 2.5%	1	F	46	158	49	10			+	8.4	
	2	F	75	159	81	15			+	9.8	
	3	F	66	149	54	22	+			7.7	
	4	F	64	148	68	10			+	6.9	
	5	F	69	144	45	19			+	9.7	
	6	M	67	160	82	2			+	6.3	
	7	M	62	160	68	3			+	5.0	
	8	F	47	155	76	3			+	10.0	
	9	F	65	152	66	18	+		+	4.8	
	10	F	58	148	63	7			+	11.8	
	$\bar{x} \pm s$		65 ± 9	153 ± 6	65 ± 13	10.9 ± 7.3				8.04 ± 2.2	
Glucagon 5.0%	1	F	67	157	57	26			+	5.8	
	2	F	72	151	65	5			+	10.2	
	3	F	56	158	64	14	+			15.2	
	4	M	58	162	66	11			+	4.6	
	5	F	73	153	69	11			+	5.6	
	6	M	39	173	85	2			+	6.1	
	7	F	65	148	50	8	+			15.2	
	8	F	63	156	71	13			+	9.4	
	9	F	60	151	57	7			+	9.1	
	10	F	58	159	47	3	+			11.3	
	11	F	54	140	53	2			+	11.8	
	$\bar{x} \pm s$		60 ± 10	155 ± 8	62 ± 11	9.3 ± 7.0				9.48 ± 3.7	
Glucagon 7.5%	1	M	73	163	70	7	+			6.9	
	2	M	50	168	68	6			+	4.8	
	3	F	65	148	60	9	+			5.4	
	4	F	50	147	70	3			+	6.8	
	5	F	60	169	73	8				10.2	
	6	M	71	153	63	30			+	8.9	
	7	F	67	159	67	21	+			8.3	
	$\bar{x} \pm s$		62 ± 9	158 ± 9	67 ± 4	12 ± 9.8				7.34 ± 1.9	

condition.

All the patients were fasted overnight. Informed consent was obtained for each patient. All patients received one drop each of PBS alone as controls or glucagon solution in both eyes in the early morning. Capillary whole blood glucose concentrations were measured with Glucometer II (Ames Co, USA) at time 0, 30, 60, and 90 min after PBS or glucagon instillation. Patients were randomly divided into 4 groups. Fifteen patients were in group 1 to receive PBS as control; 10 patients were in group 2 to receive 2.5% glucagon solution; 11 patients were in group 3 to receive 5.0% glucagon solution; and 7 patients were in group 4 to receive 7.5% glucagon solution (Tab 1).

The eye irritation was examined with standard Draize Test⁽³⁾ with 10% glucagon solution on rabbit eyes.

Data were presented as mean \pm s and repeated measurement studies were analyzed by BMDP2 V in BMDP series⁽⁴⁾.

RESULTS

Patients treated with PBS showed a gradual decrease in blood glucose (Fig 1). On the contrary, all those who received glucagon eyedrops showed significant increases in blood glucose with dose-response relationship among 2.5%, 5.0%, and 7.5% glucagon solutions. The blood glucose peaked at 30 min after glucagon instillation, came back down to halfway at 60 min; and returned to base level at 90 min. With 2.5% glucagon, the blood glucose increased $0.83 \text{ mmol} \cdot \text{L}^{-1}$ from the control level in 30 min; maintained at $0.77 \text{ mmol} \cdot \text{L}^{-1}$ above base level in 60 min; and then came down to $0.14 \text{ mmol} \cdot \text{L}^{-1}$ above control level in 90 min. When 5.0% glucagon was used, the blood glucose rose $1.76 \text{ mmol} \cdot \text{L}^{-1}$ above the baseline in 30 min; remained high at $1.52 \text{ mmol} \cdot \text{L}^{-1}$ above base level in 60 min; and

then came back down to $0.63 \text{ mmol} \cdot \text{L}^{-1}$ above control value in 90 min. With 7.5% glucagon, the blood glucose raised to $1.91 \text{ mmol} \cdot \text{L}^{-1}$ above control in 30 min; dropped quickly to $0.66 \text{ mmol} \cdot \text{L}^{-1}$ above baseline in 60 min; and then to $0.23 \text{ mmol} \cdot \text{L}^{-1}$ above base line in 90 min.

No side effect either subjectively or objectively was seen after glucagon instillation. These results coincided well with the eye irritation test with 10% glucagon eyedrops instilled into rabbit eyes which showed no irritation either.

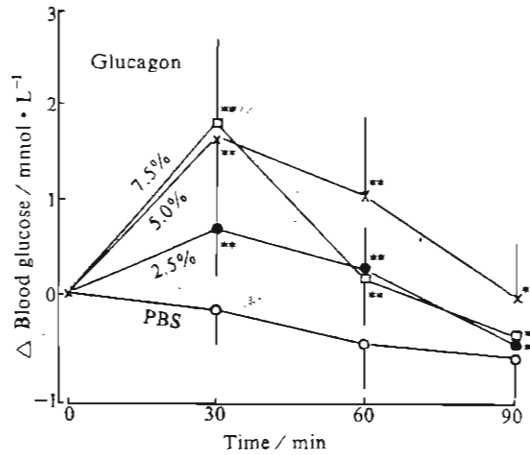


Fig 1. Effects of glucagon 2.5% ($n=10$), 5.0% ($n=11$), and 7.5% ($n=7$) eyedrops on blood glucose of fasted diabetics. Phosphate buffer saline as control ($n=15$). * $P > 0.05$, ** $P < 0.05$.

DISCUSSION

From rabbit experiments, it has been clearly shown that 0.2% of glucagon eyedrops can effectively raise the blood glucose concentrations in 30 min⁽¹⁾. In this study, similar results were observed in human patients except that the concentrations of glucagon solutions used had to be much higher, at least at 2.5%. When 7.5% of glucagon was used, the blood glucose raised $1.99 \text{ mmol} \cdot \text{L}^{-1}$ above the base level. Maximal blood glucose levels were achieved within 30 min which was close

to the reported after intravenous, intramuscular, or intranasal administrations of glucagon in healthy or diabetic subjects⁽⁵⁻¹¹⁾. It is critical that the blood glucose be peaked at 30 min after glucagon instillation because the hypoglycemia has to be corrected as soon as possible in order to avoid irreversible neurological damage and/or death^(12,13).

Comparing to the maximal blood glucose levels achieved by parenteral administration of glucagon, the levels achieved in this study was much lower. If a higher blood concentration of glucagon is needed, the concentration of glucagon eyedrop has to be raised further beyond 7.5% or absorption enhancers to be added to increase its systemic action. Absorption enhancer had been used successfully for intranasal glucagon spray to raise blood glucose level^(10,11).

The use of glucagon administration followed by oral glucose ingestion had been a treatment modality for hypoglycemia induced by insulin or sulfonylurea agents. Many patients regained consciousness sufficiently to allow them to swallow a glucose solution within 30 min of glucagon injection. However, some patients failed to benefit from glucagon⁽¹⁴⁾. In those non-responders, the mean rise in blood glucose concentration was less than $0.5 \text{ mmol} \cdot \text{L}^{-1}$ (9 mg%). This level was easily surpassed by glucagon eyedrops instillation as shown in the study. The reasons for the discrepancy of glycemic response between the responders and non-responders included the period of unconsciousness and the status of hepatic glycogen storage. Further clinical trials on the treatment modality using glucagon eyedrops will be needed to establish the usefulness of glucagon eyedrops as an alternative to treat hypoglycemic episodes in diabetic patients.

In conclusion, it is feasible to use glucagon eyedrops for the increase of blood glucose in hypoglycemic crisis in humans.

The systemic action of glucagon can be enhanced further by increasing the glucagon eyedrop concentration or by adding absorption enhancers in the eyedrops.

REFERENCES

- 1 Chiou GCY, Chuang CY. Treatment of hypoglycemia with glucagon eye drops. *J Ocular Pharmacol* 1988; 4 : 179-86.
- 2 Chuang LM, Wu HP, Chiou GCY. Treatment of hypoglycemia with glucagon eyedrops. *Pharmacologist* 1989; 31 : 125.
- 3 Draize JH, Nelson AA, Calvery HO. Percutaneous absorption of DDT in laboratory animals. *J Pharmacol Exp Ther* 1944; 82 : 159-66.
- 4 Dixon WJ, Brown MG. Biomedical Computing Programs. P Series. Berkeley: Univ of California Press, 1977.
- 5 Arky RA, Finger M, Veverbrants E, Braun AP. Glucose and insulin response to intravenous glucagon during starvation. *Am J Clin Nutr* 1970; 23 : 69-95.
- 6 Haro EN, Blum SF, Faloon WW. The glucagon response of fasting obese subjects. *Metabolism* 1965; 14 : 976-84.
- 7 Collier A, Steedman DJ, Patrick AW. Comparison of intravenous glucagon and dextrose in treatment of severe hypoglycemia in an accident and emergency department. *Diabetes Care* 1987; 10 : 712-5.
- 8 Hall-Boyer K, Zaloga GR, Chernow B. Glucagon: hormone or therapeutic agent? *Crit Care Med* 1984; 12 : 584-9.
- 9 Elrick H, Witten TA, Arai Y. Glucagon treatment of insulin reactions. *N Engl J Med* 1958; 258 : 476-80.
- 10 Pontiroli AE, Alberetto M, Pozza G. Metabolic effects of intranasally administered glucagon: comparison with intramuscular and intravenous injection. *Acta Diabetol Lat* 1985; 22 : 103-9.
- 11 Freychet L, Rizkalla SW, Desplanque N, Basdevant A, Zirinis P, Tchobroutsky G, et al. Effect of intranasal glucagon on blood glucose levels in healthy subjects and hypoglycaemic patients with insulin-dependent diabetes. *Lancet* 1988; 1 : 1364-6.
- 12 Kalimo H, Olsson Y. Effects of severe hypoglycemia on the human brain. *Acta Neur*

Scand 1980; 62 : 345-56.

13 Eeg-Olofsson O. Hypoglycaemia and neurological disturbances in children with diabetes mellitus. *Acta Paediatr Scand* 1977; 270 Suppl : 91-5.

14 MacCuish AC, Munro JF, Duncan LJP. Treatment of hypoglycaemic coma with glucagon, intravenous dextrose and mannitol infusion in a hundred diabetics. *Lancet* 1970; 2 : 946-9.

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Effects of Picroliv, the active principle of *Picrorhiza kurroa*, on biochemical changes in rat liver poisoned by *Amanita phalloides*

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ABSTRACT The efficacy of Picroliv, a standardized iridoid glycoside fraction of *Picrorhiza kurroa*, was studied against the *Amanita phalloides*-induced biochemical changes in rat liver. *A phalloides* (50 mg · kg⁻¹) caused significant increases in the activities of hepatic 5'-nucleotidase, γ -glutamyl transpeptidase, acid ribonuclease, and succinate dehydrogenase, but a decrease in glucose-6-phosphatase. The level of cytochrome P-450 in microsomal fraction and content of glycogen in liver showed significant depletions. Picroliv (25 mg · kg⁻¹ · d⁻¹ × 10 d) provided significant restorations of all the biochemical changes poisoned by *A phalloides* except cytochrome P-450 and glycogen. These results demonstrated the protective effect of Picroliv against *A phalloides*-induced hepatotoxicity in rats.

KEY WORDS *Amanita*; *Picrorhiza kurroa*; Picroliv; liver; enzyme tests; cytochrome P-450; liver glycogen

Picroliv (Pic) is a standardized iridoid glycoside fraction isolated from roots and

rhizomes of *Picrorhiza kurroa*. It contains 60% picroside I and kutkoside in a ratio of 1 : 1.5, the balance being minor constituents. Earlier studies have shown the hepato-protective efficacy of Pic against CCl₄-⁽¹⁾, galactosamine-⁽²⁾, monocrotaline-⁽³⁾, paracetamol-⁽⁴⁾, and thioacetamide-⁽⁵⁾ induced liver damages in rats, and *Plasmodium berghei* infection in *Mastomys*⁽⁶⁾.

A phalloides (AP) is the most toxic mushroom causing 95% of total mushroom poisonings. It contains mainly 2 toxins : amanitin and phalloidin. Phalloidin is the major toxic agent and is known to act on plasma membrane of hepatocytes in rats⁽⁷⁾. However, the mortality of rats poisoned with AP was reduced by Pic⁽⁸⁾. In this communication we have reported the influence of Pic on biochemical changes poisoned by AP in rat liver.

MATERIALS AND METHODS

Adult ♂ rats (wt 130 ± s 15 g) of Sprague-Dawley strain, inbred in CDRI Animal House were used. The rats were fed *ad lib* standard pellet diet (Lipton, Bombay) and allowed free access to water.

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