

Development of a Novel Anti-Diabetes Agent: Clinical Study

Moon K. Song, Ph.D., Senior Research Scientist

Department of Pediatrics, UCLA School of Medicine, Le Conte Ave, Los Angeles, CA 90095, USA and
Research Service, VA Greater Los Angeles Healthcare System, Wilshire Blvd, Los Angeles, CA 90073, USA
Conducted at Yan Bian Hanmi Medical Center, Yanbian, China, 2007 - 2008

ABSTRACT

Used inactive insulin in the cells induces insulin resistance by interfering insulin signaling process for glucose uptake. Insulin degrading enzyme (IDE) is a zinc enzyme, which degrades internalized inactive used insulin. Pro-Z compound enhances zinc metabolism and CHP (cyclo his-pro) plus zinc (Pro-Z) treatment significantly increased IDE synthesis and muscle glucose uptake in animals. More importantly, treatment of diabetic animals with Pro-Z significantly decreased blood glucose and plasma insulin levels as well as improving oral glucose tolerance. Based on these facts, we performed FDA standard phase 1 clinical trial and a pilot study with 18 diabetic subjects to determine the effects of Pro-Z (containing 3 mg CHP, excipient, plus 20 mg zinc) on the changes of blood glucose, Hemoglobin A1c and insulin doses for 180 days. No side effects were observed with acute treatment with 4 folds of optimal doses of Pro-Z in 12 normal healthy subjects. These subjects also maintained significantly lower blood glucose levels than fasting blood glucose levels for more than 8 hours even after the breakfast and lunch. In the pharmacokinetic study, plasma CHP concentration was highest at 4 hours after the intake of Pro-Z and maintained higher levels for 24 hours than the baseline level. Diabetic subjects treated with Pro-Z for 6 months exhibited extremely significant decreases of blood glucose and Hemoglobin A1c levels, and their insulin doses. These pre-clinical studies with various animal models and clinical trials with healthy and diabetic subjects strongly support potentiality of Pro-Z to become one of the most important global anti-diabetes agents.

Key words: Diabetes Mellitus, Anti-diabetes agent, Cyclo (his-pro), Zinc, Human study

INTRODUCTION

Insulin degrading enzyme (IDE) is a zinc enzyme and plays a major role in the regulation of internalized insulin degradation (1). However, diabetic animals and humans are zinc deficient (2) due to impaired intestinal zinc absorption and hyperzincuria (3). Zinc deficiency causes decreased IDE synthesis, which may induce hyperinsulinemia and insulin resistance by decreasing digestion of internalized insulin (1). Cyclo (his-pro) (CHP) stimulates intestinal zinc absorption and cellular zinc uptake (4), and glucose uptake (5). Pro-Z (CHP plus zinc) enhances IDE synthesis and stimulates degradation of used inactive insulin in the cells. Although CHP or zinc alone is somewhat effective in the control of blood glucose metabolism, only Pro-Z significantly improved oral glucose tolerance (OGT), and decreased blood

glucose and plasma insulin levels in animals (5,6). The present study demonstrated that Pro-Z ameliorates insulin resistance and diabetes in humans.

MATERIALS AND METHODS

For FDA standard Phase I clinical trial, approximately 60 health subjects were recruited and divided into 4 groups of about 15 subjects per group. All the subjects were given either 8 capsules of placebo, 2 capsules of Pro-Z containing 3 mg CHP plus 20 mg zinc and 6 capsules of placebo, 4 capsules of Pro-Z and 4 capsules of placebo, or 8 capsules of Pro-Z before breakfast. Blood samples were drawn at baseline, 2 hours, 4 hours, 8 hours and 24 hours, and analyzed for lipid, kidney and liver panels for toxicity determination, and plasma CHP concentration for measurement of pharmacokinetics of CHP. Eighteen diabetic subjects with fasting blood glucose 6.5 mmol/L or greater, who meet all inclusion and exclusion criteria, were invited to participate in the study. If a subject was willing to adhere to the study protocol for 6 months, the subject was enrolled into the study. These volunteers were then given 60 capsules of Pro-Z containing 3 mg CHP plus 20 mg zinc to consume 2 capsules every day just before bed time. All subjects were instructed in a lifestyle modification including a hypocaloric balanced diet and maintaining a daily log of their fasting and postprandial blood glucose levels 2 hours after breakfast, lunch, and dinner, and their drug dosages. Patients were told to reduce insulin injection or/and oral agent doses whenever they experience hypoglycemia, to notify their physician if they feel adverse effect, and to come in for a physical examination if necessary. At each visit every 30 days, the subjects were examined, given a Pro-Z bottle, and their records of drug dosages and blood glucose measurements were collected. At each visit, hemoglobin A1c levels were measured and given physical examinations for all participants

RESULTS

The double-blinded study showed no adverse side effects in subjects taking one time oral intake of 0, 2, 4, or 8 capsules of Pro-Z. All subjects had normal blood chemistry data and cell numbers at the start of the trial (0 hours) and no significant changes of these data from the baseline were exhibited during 24 hour study period (0, 2, 4, 8, and 24 hours). However, subjects (n = 12) who took 8 capsules of Pro-Z before breakfast showed significantly reduced plasma glucose levels at 8 hours, but their plasma glucose levels were within normal range (Table 1). Twenty-four hours later, the blood glucose levels returned to the similar levels prior to Pro-Z intake. This dose was only 70-80% of the acute optimal dose of Pro-Z shown on diabetic animals to improve oral glucose tolerance. Plasma CHP concentrations were highest after 4 hours of Pro-Z intake. However 4 folds of optimal doses 8 capsules of Pro-Z intake increased only 50 % intake of 2 capsules (Fig. 1). This shows that the higher amount of Pro-Z intake than the optimal dose of Pro-Z intake would not help in improving diabetes. Furthermore, it shows that after 24 hours there were essentially no consumed CHP left in the plasma, which indicates further that CHP intake is safe since it is rapidly metabolized.

Table 1 Changes of Plasma Glucose levels in Phase 1 Clinical Trial

Pro-Z capsule	0 hours		2 hours		4 hours		8 hours		24 hours		P-values
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
0	<u>106.5</u>	2.83	<u>102.5</u>	4.25	<u>99.1</u>	3.40	<u>99.3</u>	2.86	<u>102.2</u>	3.76	NS
2	<u>97.6</u>	2.43	<u>96.5</u>	3.68	<u>95.2</u>	3.39	<u>95.8</u>	8.93	<u>94.8</u>	5.17	NS
4	<u>92.4</u>	5.05	<u>91.9</u>	4.37	<u>94.2</u>	7.63	<u>90.9</u>	5.63	<u>97.9</u>	4.66	NS
8	<u>107.6</u>	5.91	<u>91.7</u>	6.26	<u>92.6</u>	2.83	<u>88.3</u>	2.44	<u>103.9</u>	7.13	0.0495*

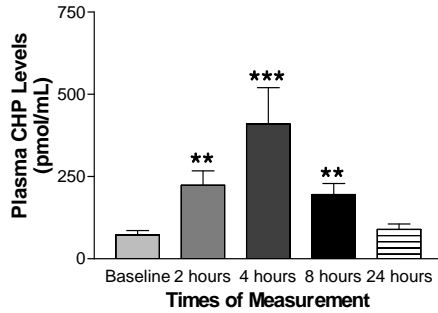


Fig. 1A Treated with 2 capsules of Pro-Z

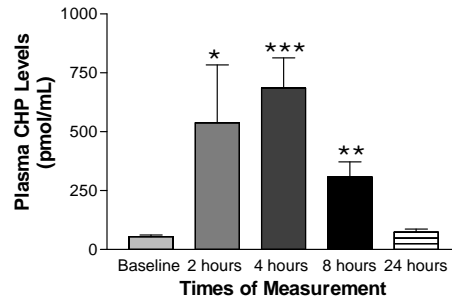


Fig. 1B Treated with 8 capsules of Pro-Z

During 6 month treatment period, fasting blood glucose levels slightly increased for 60 days, then started to decrease as shown in Table 2. However, no statistically significant decrease was shown with fasting blood glucose levels. In contrast, decreases of postprandial blood glucose levels two hours after breakfast, lunch and dinner were all extremely significant ($p < 0.0001$, < 0.0001 and 0.0003 respectively). There were slight increases of all postprandial blood glucose levels after 120 day treatment. This is probably due to the decrease of insulin injections or oral agent doses. Hemoglobin A1c levels, however, more significantly decreased during 90-180 day treatment period ($p < 0.01$) than the first 90 day treatment period ($p < 0.05$) (Fig. 1). These facts imply that glucose tolerance is improved in these patients. More interestingly, decreases of insulin doses before breakfast, lunch and dinner were all extremely significant ($p < 0.0001$) (Fig. 2). Essentially all the study subjects either totally stopped insulin injection or used very low doses of insulin at the end of 6 month study. Metformin dose decreases in three subjects were 2.688 ± 0.805 mg/day with p-value 0.079. The drug dosage decreases in 6 subjects were not determined since they were either naïve to the drug treatment, used different oral agents irregularly, or used unknown drugs. However, the drug dosages in these subjects showed a tendency to decrease. Most of patients progressively reduced their pre-Pro-Z treatment anti-diabetes agents. Neither incidence of hypoglycemia nor other adverse side effect was reported during this study period.

Table 2 Mean Monthly Average Blood Glucose Level Changes during Six-months Pro-Z Treatment

Treatment	Fasting	After Breakfast	After Lunch	After Dinner
Days	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
-15-0	7.23 ± 0.31	10.59 ± 0.64	10.63 ± 0.48	10.29 ± 0.72
0-30	7.27 ± 0.33	9.53 ± 0.34	9.57 ± 0.30 ***	9.07 ± 0.34
30-60	8.12 ± 0.47	8.80 ± 0.34*	8.53 ± 0.23***	8.55 ± 0.30*
60-90	7.52 ± 0.32	8.21 ± 0.27***	8.05 ± 0.30 ***	7.85 ± 0.25 ***
90-120	7.11 ± 0.29	7.84 ± 0.30***	7.86 ± 0.27***	8.01 ± 0.28 **
120-150	7.08 ± 0.32	8.28 ± 0.29***	8.19 ± 0.33***	8.16 ± 0.31**
150-180	7.46 ± 0.42	8.79 ± 0.37*	8.31 ± 0.34 ***	8.39 ± 0.32 *

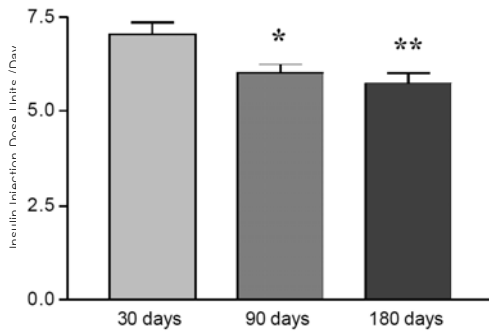


Fig.2 Mean hemoglobin A1c levels of 18 diabetic subjects measured at 20, 90, and 180 days after the initiation of Pro-Z treatment. *p<0.05; **p<0.01 compared to the 30-day treatment values.

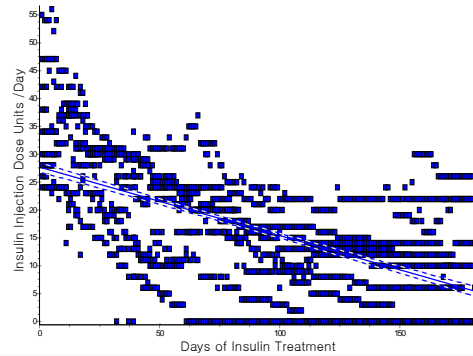


Fig. 3 Daily insulin dose records while treated with Pro-Z. The decreasing rate of insulin injection dose was -0.1126 ± 0.0042 units/day with $p<0.0001$.

DISCUSSIONS

Our preclinical studies with animal models indicated that the optimal daily dose for lowering blood glucose level was 0.5 mg CHP plus 10 mg zinc/L (CZ) in the drinking water for genetically diabetic G-K rats (5) and obese diabetic *ob/ob* mice (6). This dose is equivalent to 0.1mg CHP plus 2 mg zinc/Kg BW/day. Plasma insulin levels in G-K rats significantly increased and blood glucose levels slightly decreased by treatment with either CHP or zinc alone. However, CZ treatment very significantly decreased both blood glucose and plasma insulin levels (5, 6). The key finding in these studies is that plasma insulin levels decreased in CZ treated rats, while oral glucose tolerance and blood glucose levels decreased. These facts suggest that CZ treatment improves insulin resistance or ameliorates diabetes. In the present study, Pro-Z treatment very significantly decreased postprandial blood glucose levels (Table 1) and HbA1c levels (Fig. 1) while insulin injections doses were decreased (Fig. 3) in humans. These facts support our hypothesis that Pro-Z treatment improves insulin resistance and diabetes in humans. The possible mechanism by which Pro-Z improves insulin resistance is stimulation of insulin sensitivity by enhancing IDE synthesis.

Fig. 1 Daily insulin dose records while treated with Pro-Z

CONCLUSIONS

The present study with Pro-Z strongly supports the potentiality that Pro-Z ameliorates insulin resistance and diabetes in humans, which will lead to the development of diabetes and pre-diabetes (metabolic syndrome) supplemental compound for global commercialization.

REFERENCES

- [1] Mora, M. E.V., Searfone, A., Calvani, M., Greco, A.V. and Mingrone G. 2004. Insulin clearance in obesity. *J. Am. Coll. Nutr.* 22: 4897-493.
- [2] Aguilar, M.V., Laborda, J.M., Martines-Para, M.C., Gonzalez, M.J., Mesquer, I., Bernao, A. and Mateos, C.J. 2004. Effects of diabetes on the tissue Zn/Cu ratio. *J. Trace Elem. Med. Biol.* 12:155-158.
- [3] Kinlaw, W.B., Levine, A.S., Moreley, J.E., Silvis, S.E. and McClain, C.J. 1983. Abnormal zinc metabolism in Type II diabetes mellitus. *Am J Med* 75:273-77, 1983.
- [4] Rosenthal, M.K., Hwang, I.K. and Song, M.K. 2001. Effects of arachidonic acid and cyclo (his-pro) on zinc transport across small intestine and muscle tissues. *Life Sci.*70:337-348
- [5] Song, M.K., Hwang, I.K., Rosenthal, M.J., Harris, D.M., Yamaguchi, D.T. and Go, V.L.W. 2003. Anti-hyperglycemic activity of zinc plus cyclo (his-pro) in genetically diabetic Goto-Kakizaki and aged Rats. *Exp. Biol. Med.* 228:1338-1345.
- [6] Hwang, I.K., Go V.L.W., Harris, D.M., Yip, I., Kang, K.W. and Song M.K. 2003. Effects of cyclo (his-pro) plus zinc on glucose metabolism in genetically diabetic obese mice. *Diabet. Obe. Metabol.* 5:317-324.