Anti-diabetes Effect of Zn(II)/Carnitine Complex by Oral Administration

Yutaka YOSHIKAWA,[a] Eriko UEDA,[a] Hiromu SAKURAI,[b] and Yoshitane KOJIMA[a,]*

[a] *Department of Chemistry, Graduate School of Science, Osaka City University; 3–3–138 Sugimoto, Sumiyoshi-ku, Osaka 558–8585, Japan: and* [b] *Department of Analytical and Bioinorganic Chemistry, Kyoto Pharmaceutical University; 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607–8414, Japan.*
Received October 18, 2002; accepted December 17, 2002; published online December 24, 2002

A novel bis(L-carnitinato)Zn(II) complex, $Zn(car)_2Cl_2$, was prepared, and its insulinomimetic and antidiabetic activities were examined. The complex showed a tendency to lower the high blood glucose levels of KK-A^y mice with type 2 diabetes mellitus when given by oral administration at a dose of 20 mg Zn/kg body weight for 16 d. In addition, the complex improved glucose tolerance ability when examined by the oral glucose tolerance test (1 g glucose/kg body weight).

Key words zinc(II) complex; L-carnitine; hypoglycemic effect; glucose tolerance test; type 2 diabetes; oral administration

Recently, both Zn(II) ion and several Zn(II) complexes have been found to have insulinomimetic activity.[1)] We reported that Zn(II) complexes with nicotinamide, maltol, amino acids, picolinic acid, picolinamide, and their derivatives have high insulinomimetic activities in *in vitro* and *in vivo* studies.[2—7)] However, these complexes were effective in showing the lowering effect of blood glucose by daily intraperitonial injections to type 2 diabetes mellitus. When aiming at the clinical use of the complexes, it is essentially important to find complexes that are effective on oral administration. During the investigation for developing orally active insulinomimetic Zn(II) complexes, we found that a Zn(II) complex with L-carnitine ((*R*)-3-hydroxy-4-(trimethylammonio)butanoate : car) improves type 2 diabetes mellitus in mice when administered orally.

L-Carnitine is known to be a vitamin-like nutrient (Vitamin B_T) found in the heart, brain, and skeletal muscles.[8—10)] It was reported that L-carnitine is incorporated in the body through the carnitine transporter.[11,12)] Its primary physiological role is to transport fatty acids across the cell wall into the mitochondria to provide the heart and skeletal cells with energy.[13)] In *in vitro* studies, it was reported that L-carnitine reverses much of the damage inflicted on the brain by free radicals.[14)] L-Carnitine has been used as a supplement which maintains physical stamina and promotes weight loss.[15)] On the other hand, the body uses L-carnitine to produce the enzyme, acetyl-L-carnitine transferase, which boosts choline metabolism and releases acetylcholine in the brain.[16,17)]

We synthesized the complex, $Zn(car)_2Cl_2$, by the following method. To an aqueous solution of L-carnitine (30 mmol), an aqueous solution of $ZnCl_2$ (15 mmol) was added and stirred for 0.5 h at room temperature. After the solution was uniform, the solvent was evaporated under the reduced pressure. The obtained oily residue was dissolved with ethanol and the solution was evaporated under the reduced pressure. The obtained powder was filtered off, washed with ethanol, and dried *in vacuo*. Yield: 63%. *Anal.* Found (%): C, 35.73; H 6.78; N 5.70. Calcd (%) for $Zn(C_7H_{15}NO_3)_2Cl_2 \cdot 0.5H_2O$; C, 35.95; H 6.68; N 5.99. IR (KBr disk): 1610 cm^{-1} for $\nu_{C=O}$. mp 83—90 °C. $[\alpha]_D^{20}$ +3.2° (H_2O). On the other hand, IR spectra of the C=O stretching with L-carnitine at 1601 cm^{-1} have shifted to higher frequencies, 1610 cm^{-1} ($\nu_{C=O}$ for $Zn(car)_2Cl_2$). Therefore, we estimated the structure of $Zn(car)_2Cl_2$ as shown in Fig. 1.

The insulinomimetic activity of the complex was evaluated by using the isolated rat adipocytes treated with epinephrine in terms of the inhibition of free fatty acid release.[18,19)] The inhibitory effect of $Zn(car)_2Cl_2$ was compared with those of $VOSO_4$ and $ZnSO_4$ as positive controls (Fig. 2).

The effects of the three compounds were dose-dependent in the concentration range of 10^{-4}—10^{-3} M. From these results, the apparent IC_{50} value, a 50% inhibitory concentration of the FFA released in each complex, was estimated to be 0.80 ± 0.05 mM for $Zn(car)_2Cl_2$. $Zn(car)_2Cl_2$ was found to be more active than $VOSO_4$ ($IC_{50}=1.00 \pm 0.08$ mM) and to have almost the same activity to that of $ZnSO_4$ ($IC_{50}=0.81 \pm 0.10$ mM). In our previous research, if IC_{50} value of the VO compounds were equal to or less than 1.00 mM, the compounds showed antidiabetic activity by oral administration.[1)] In this study, we tested the Zn(II) complex by oral administration.

We evaluated the *in vivo* blood glucose lowering effect of $Zn(car)_2Cl_2$ in KK-A^y mice (22 weeks old: CREA Japan Inc., Tokyo) with type 2 diabetes mellitus. In this study, we used KK-A^y mice at the age of 22 weeks old because the blood glucose levels of old diabetes patients were reduced by administration of bovine prostate powder supplemented with Zn.[20)] They received oral administration of the complex daily at about 10:00 a.m. for 16 d. Blood samples were obtained from the mouse-tail vein, and the glucose levels were measured with a Glucocard (Arkray, Kyoto). The body weights of KK-A^y mice, which were allowed free access to solid food

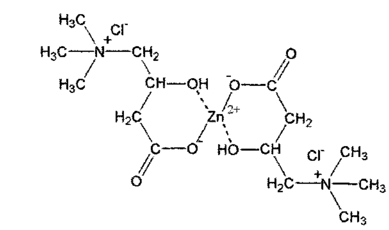

Fig. 1. Estimated Structure of Bis(L-carnitinato)Zn(II) Complex

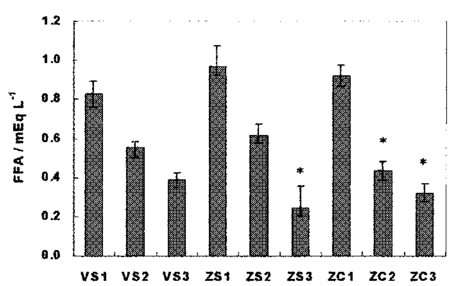

Fig. 2. Inhibitory Effects of $VOSO_4$ (VS), $ZnSO_4$ (ZS), and $Zn(car)_2Cl_2$ (ZC) on Free Fatty Acids Released from Rat Adipocyte Treated with Epinephrine

Concentrations of VS1, ZS1, and ZC1 were 10^{-4} M, those of VS2, ZS2, and ZC2 were 5×10^{-4} M, and those of VS3, ZS3, and ZC3 were 10^{-3} M. * Significance at $p<0.05$ *vs.* the $VOSO_4$ (by Student's *t*-test).

* To whom correspondence should be addressed. e-mail: kojima@sci.osaka-cu.ac.jp

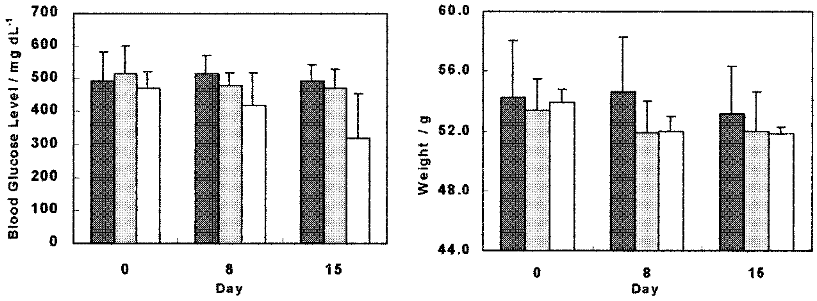

Fig. 3. Changes of Blood Glucose Level (Left) and Body Weight (Right) in the Control KK-A^y Mice (■), the KK-A^y Mice Treated with L-Carnitine (■) and the KK-A^y Mice Treated with $Zn(car)_2Cl_2$ (□)

Each column is expressed at the mean ±S.D. for three or four mice.

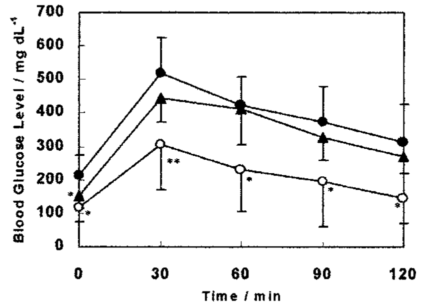

Fig. 4. Oral Glucose Tolerance Tests for the Control KK-A^y Mice Receiving Daily Oral Administration of Water (Control: ▲), L-Carnitine (●) and $Zn(car)_2Cl_2$ (○)

Oral glucose tolerance tests were performed on mice that fasted for 12 h, then given glucose solution orally at a dose of 1 g/kg body weight. Symbols are means ±S.D. for three or four mice. ** Significance at $p<0.01$ *vs.* the KK-A^y mice treated with L-carnitine (by Student's *t*-test). * Significance at $p<0.05$ *vs.* the KK-A^y mice treated with L-carnitine (by Student's *t*-test).

(CREA Japan Inc.) and tap water, were measured daily during the administration of $Zn(car)_2Cl_2$. The intakes of solid food and drinking water for each mouse were checked daily throughout the experiments. When the mice were given $Zn(car)_2Cl_2$ at a dose of 20 mg Zn/kg body weight, they had a tendency to lower the blood glucose levels during the complex administration for 16 d (Fig. 3 left). The body weight of the KK-A^y mice in each group decreased slightly (Fig. 3 right). Also, due to the improvement of the blood glucose level of the KK-A^y mice treated with the $Zn(car)_2Cl_2$ by daily oral administrations at the dose of 20 mg Zn/kg for 16 d, the KK-A^y mice received the oral glucose tolerance test. As shown in Fig. 4, the blood glucose levels of the KK-A^y mice treated with L-carnitine were elevated to the maximum (520 mg/dl=28.9 mM) at 30 min after the glucose administration, and then gently decreased. In contrast, the blood glucose levels of the KK-A^y mice treated with $Zn(car)_2Cl_2$ were also elevated (304 mg/dl=16.9 mM) for 30 min, however, they were obviously lowered when compared with those of the KK-A^y mice treated with L-carnitine. The area under the curves (*AUC*) of the glucose level in the blood were calculated from the data in Fig. 4, being estimated to be 690±154, 789±184 and 431±216* (mg/dl h) for the control KK-A^y mice, KK-A^y mice treated with L-carnitine, and KK-A^y mice treated with $Zn(car)_2Cl_2$, respectively (* significance at $p<0.05$ *vs.* the KK-A^y mice treated with L-carnitine (by Student's *t*-test)). These results strongly indicated that the treatment of $Zn(car)_2Cl_2$ improves type 2 diabetes mellitus in KK-A^y mice by oral administration.

The proposed $Zn(car)_2Cl_2$ is assumed to be incorporated in the body through the carnitine transporter. Previously, Shisheva et al. have reported that a high dose of $ZnCl_2$ (210 mg Zn/kg body weight) stimulates the glucose uptake in rat adipocytes and reduces blood glucose concentration as much as 50% when was given orally to STZ rats.[21] In contrast, $Zn(car)_2Cl_2$ at a low dose (20 mg Zn/kg body weight) was effective in lowing the blood glucose concentration orally administered.

In conclusion, $Zn(car)_2Cl_2$ has been found to have the *in vitro* insuinomimetic activity and *in vivo* blood glucose lowering effect, the improvement of the diabetes being supported by the results of the glucose tolerance test. The present result is the first example for the orally active Zn(II) complex. A detailed study about the mechanism of $Zn(car)_2Cl_2$ is now under way. The obtained results will be useful when they are clinically used on type 2 diabetes mellitus in the future.

The authors are grateful to the members of the analytical center of Osaka City University for elemental analyses.

References and Notes

1) Sakurai H., Kojima Y., Yoshikawa Y., Kawabe K., Yasui H., *Coord. Chem. Rev.*, **226**, 187—198 (2002).
2) Yoshikawa Y., Ueda E., Kawabe K., Miyake H., Sakurai H., Kojima Y., *Chem. Lett.*, **2000**, 874—875 (2000).
3) Ueda E., Yoshikawa Y., Kishimoto N., Tadokoro M., Yanagihara N., Sakurai H., Kojima Y., *Chem. Lett.*, **2001**, 1184—1185 (2001).
4) Yoshikawa Y., Ueda E., Miyake H., Sakurai H., Kojima Y., *Biochem. Biophys. Res. Commun.*, **281**, 1190—1193 (2001).
5) Yoshikawa Y., Ueda E., Suzuki Y., Yanagihara N., Sakurai H., Kojima Y., *Chem. Pharm. Bull.*, **49**, 652—654 (2001).
6) Yoshikawa Y., Ueda E., Kawabe K., Miyake H., Takino T., Sakurai H., Kojima Y., *J. Biol. Inorg. Chem.*, **7**, 68—73 (2002).
7) Ueda E., Yoshikawa Y., Ishino Y., Sakurai H., Kojima Y., *Chem. Pharm. Bull.*, **50**, 337—340 (2002).
8) Bremer J., *Physiol. Rev.*, **63**, 1420—1480 (1983).
9) Lai H. S., Chen Y., Chen W. J., *World J. Surg.*, **22**, 42—46 (1998).
10) Redouche C. J., Seim H., *Ann. Rev. Nutr.*, **18**, 39—61 (1998).
11) Satoh S., *Ryoikibetsu Shokogun Shirizu*, **18 Pt1**, 398—400 (1998).
12) Matsuda K., Yuasa H., Watanabe J., *Biol. Pharm. Bull.*, **21**, 752—755 (1998).
13) Jeukendrup A. E., *Ann. N.Y. Acad. Sci.*, **967**, 217—235 (2002).
14) Koudelova J., Mourek J., Drahota Z., Rauchova H., *Physiol. Res.*, **43**, 387—389 (1994).
15) Mindell E., "Supplement Bible," Simon & Schuster, New York, 1998, pp. 193—194.
16) Arockia Rani P. J., Panneerselvam C., *Exp. Gerontol.*, **36**, 1713—1726 (2001).
17) Mindell E., "Supplement Bible," Simon & Schuster, New York, 1998, pp. 85—86.
18) Nakai M., Watanabe H., Fujisawa C., Kakegawa H., Satoh T., Takada J., Matsushita R., Sakurai H., *Biol. Pharm. Bull.*, **18**, 719—725 (1995).
19) Isolated male rat adipocytes (1.0×10^6 cells/ml) prepared as described in ref. 18 were preincubated at 37 °C for 30 min with various concentrations (10^{-4}—10^{-3} M) of zinc(II) complexes in KRB buffer (120 mM NaCl, 1.27 mM $CaCl_2$, 1.2 mM $MgSO_4$, 4.75 mM KCl, 1.2 mM KH_2PO_4, 24 mM $NaHCO_3$ and 5 mM glucose: pH 7.4) containing 2% BSA. A 10^{-4} M epinephrine was then added to the reaction mixtures and the result solutions were incubated at 37 °C for 180 min. The reactions were stopped by soaking in ice water and the mixtures were centrifuged at 3000 rpm for 10 min. For outer solution of the cells, FFA levels were determined with an FFA kit (Wako).
20) Song M. K., Rosenthal M. J., Naliboff B. D., Phanumas L., Kang K. W., *Metabolism*, **47**, 39—43 (1998).
21) Shisheva A., Gefel D., Schechter Y., *Diabetes*, **41**, 982—988 (1992).