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ScienceDirect
Food Chemistry 104 (2007) 45–52
Food Chemistry
www.elsevier.com/locate/foodchem

Improvement of insulin resistance by Hon-Chi in fructose-rich chow-fed rats

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Received 5 October 2005; received in revised form 11 April 2006; accepted 29 October 2006

Abstract

In an attempt to develop new substances for handling insulin resistance, the effect Hon-Chi was examined on insulin resistance induced by fructose-rich chow in rats. Mandarin Hon-Chi is red yeast rice fermented with *Monascus pilous* and *Monascus purpureus*. Single oral administration of Hon-Chi for 90 min decreased the plasma glucose in a dose-dependent manner in rats, which had received four-week fructose-rich chow. The insulin action on glucose disposal rate using the glucose–insulin index, and the value of the areas under the curve of glucose and insulin during the intraperitoneal glucose tolerance test were measured. Oral administration (three times daily for three-days) of Hon-Chi into rats, which received four-week fructose-rich chow, reversed the elevated value of glucose-insulin index, indicating Hon-Chi has an ability to improve insulin resistance. The time for the loss of plasma glucose lowering response to tolbutamide in fructose-rich chow-fed rats was markedly delayed by the repeated treatment of Hon-Chi, as compared to the vehicle-treated group. This provided the supportive data that oral administration of Hon-Chi could delay the development of insulin resistance in rats. Increase of insulin sensitivity by Hon-Chi was further identified using the plasma glucose lowering action of exogenous insulin in streptozotocin-induced diabetic rats (STZ-diabetic rats). Oral administration of Hon-Chi at 150 mg/kg three times daily into STZ-diabetic rats caused an increase in the responses to exogenous insulin 15-days later. The obtained results suggest that oral administration of Hon-Chi has the ability to improve insulin sensitivity and delay the development of insulin resistance in rats, which may be used as an adjuvant therapy to patients with insulin resistance.

Keywords: Fructose-rich chow; Insulin resistance; Intraperitoneal glucose tolerance test; Hon-Chi

1. Introduction

Monascus purpureus and *Monascus pilosu* are representatives of *Monascus* fungi, traditionally used in Asia as a source of food colorants (Chen, 1987). Red yeast rice, also known as "red Koji" in Japanese or "Hon-Chi" in Mandarin, produced by fermenting *Monascus* fungi on steamed rice, has been used for more than 600 years to produce wines and other fermented food (Fabre et al., 1993). Also, Hon-Chi has been widely recognized in China as a folk medicine for improving digestion and blood circulation (Journoud & Jones, 2004; Ma et al., 2000).

Insulin resistance is a key feature of impaired glucose tolerance in type 2 diabetes, which can be characterised by a diminished ability of insulin sensitive tissues and a marked decrease of glucose metabolism in response to insulin (Kruszynska & Olefsky, 1996). Primary treatment goals in diabetes include restoration and maintenance of normoglycaemia, avoidance of diabetic complications, and prevention of cardiovascular events (Younis, Soran, & Farook, 2004).

Recent clinical observations show that Hon-Chi has the ability to lower blood lipid levels, not only in animal models but also in humans (Journoud & Jones, 2004; Ma et al., 2000). This observation is mainly due to the action of the

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0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.10.070

active principle, monacolin, in Hon-Chi, which inhibits 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase (Heber et al., 1999). Thus, it seems that Hon-Chi may reduce cardiovascular complications. However, the effect of Hon-Chi on insulin resistance, which is widely associated with cardiovascular complications, is still unknown and is investigated in the present study.

2. Materials and methods

2.1. Materials

The powder of Hon-Chi was obtained from Yusheng Pharmaceutical Co., Ltd. (Taichung, Taiwan). This commercial preparation is made by traditional methods of fermenting moistened premium rice with *M. pilous* and *M. purpureus*, followed by air-drying to yield the Hon-Chi. Streptozotocin (STZ) and tolbutamide were purchased from Sigma-Aldrich, Inc. (Saint Louis, MO). The commercial kit for enzyme-linked immunosorbent assay, to measure plasma insulin, was from Penisula Lab., Inc. (Belmont, CA). Long-acting human insulin and short-acting human insulin were the products of Novo Nordisk A/S (Bagsvaerd, Denmark). Metformin (Glucophage®) was obtained from Lipha (UK).

2.2. Animals

Male Wistar rats aged eight-weeks were obtained from the Animal Centre of the National Cheng Kung University Medical College. They were maintained in a temperature-controlled room (25 ± 1 °C) and kept on a 12:12 light-dark cycle (light on at 06:00 h). Food and water were available *ad libitum*. The rats were divided into three experimental groups. One group of rats received an intravenous injection of STZ (60 mg/kg) after fasting for three-days. Rats with plasma glucose concentration of 400 mg/100 ml or greater, in addition to polyuria and other diabetic disorders, were considered as type-1 diabetic animals. All studies were carried out two-weeks after the induction of diabetes. The other two groups of Wistar rats were not treated with STZ. One group was randomly assigned to receive fructose-rich chow (Teklad, Madison, WI) containing 60% fructose for 4 additional weeks, to induce insulin resistance, as described previously (Liu, Liu, & Cheng, 2005). The remaining rats, receiving standard chows (Purina Mills, LLC, St. Louis, MO) during the four-week period, were the control group. All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institute of Health, as well as the guidelines of the Animal Welfare Act.

2.3. Single treatment of Hon-Chi to fructose-rich chow fed rats

The powder of Hon-Chi was dissolved in saline solution, for oral administration at desired doses. The fasting rats, that received fructose-rich chow for four-weeks, received an oral administration of Hon-Chi at the desired doses or the same volume of saline solution. Blood samples (0.1 ml) of the treated rats were collected under sodium pentobarbital anaesthesia (30 mg/kg, intraperitoneal injection (IP)) from the tail vein, at the indicated time point for measurement of plasma glucose. The concentration of plasma glucose was measured by the glucose oxidase method, using an analyser (Quik-Lab, Ames, Miles Inc., Elkhart, IN).

2.4. Measurement of glucose–insulin index

The rats that were fed with four-week fructose-rich chow were used as insulin-resistant animals. The Hon-Chi dissolved in saline solution was used for oral administration at the desired doses, three times daily, into each group of the fructose-rich chow-fed rats. Another group of the fructose-rich chow-fed rats and the standard chow-fed group received similar treatments with the same volume of saline used to dissolve the Hon-Chi. In the preliminary experiments, Hon-Chi was found to improve the fructose-induced decrement of insulin-stimulated glucose disposal rate in a dose-dependent manner from 25 mg/kg to 150 mg/kg after three-days treatment.

The intraperitoneal glucose tolerance test (IPGTT) was performed in the fructose-rich chow-fed rats, which received a three-days oral administration of Hon-Chi. Then, the body weight and the daily amount of food intake and water intake in Hon-Chi treated rats were measured at the end of treatment, to compare with the saline-treated control. Fasting animals were food-restricted and given only water to drink the night before the experiment.

On the morning of IPGTT, fasting animals were used for IP injection of glucose (1 g/kg). Blood glucose levels were determined using blood samples from the tail vein taken at 0 (before glucose injection), 30, 60, 90 and 120 min after glucose administration. The blood sample was thoroughly mixed with 10 IU heparin and centrifuged to obtain the plasma. Concentration of plasma glucose was measured by the glucose oxidase method *via* an analyser (Quik-Lab), with samples run in duplicate. Enzyme-linked immunosorbent assay was carried out to measure plasma insulin, using the commercial kit specific for rats. Glucose-insulin index was calculated as the product of the glucose and insulin areas under the curve (AUC), as described previously (Liu et al., 2005).

2.5. The induction of insulin resistance in rats fed with fructose-rich chow

Wistar rats received oral administration of Hon-Chi at the highest dosage of 150 mg/kg, every 8 h, three times daily, and the others received similar treatment with the same volume of saline during the fructose-rich chow-feeding period. Plasma glucose levels were mea-

sured in tail blood samples obtained at 1:00 p.m. after a 4-h fasting (starting from 9:00 a.m.) on the indicated days.

Formation of insulin resistance in fructose-rich chow-fed rats was characterised by the loss of tolbutamide-induced plasma glucose lowering action. In brief, rats received an IP injection of 10 mg/kg tolbutamide at 5 h after treatment with red yeast rice or vehicle on the indicated date, as described previously (Liu et al., 2005). Effects on plasma glucose were determined using the blood samples collected from the tail vein of rats under anaesthesia of sodium pentobarbital (30 mg/kg, IP) at 1 h after tolbutamide injection, to characterise the formation of insulin resistance. Concentration of plasma glucose was measured as described above. Changes in the body weight and the daily food behaviour in fructose-rich chow-fed rats with or without Hon-Chi treatment were also measured during the insulin resistance induction period.

2.6. Effect on insulin sensitivity

STZ-diabetic rats were used to investigate the response to exogenous insulin. These rats received an IP injection of long-acting human insulin at 1 IU/kg once daily to normalise the insulin sensitivity (Wu et al., 2002). Then, three-days later, the STZ-diabetic rats were divided into two groups for experiment. One group of STZ-diabetic rats received an oral administration of Hon-Chi at 150 mg/kg, three times daily, and the other group received a similar treatment with the same volume of saline. The injection of long-acting human insulin at 1 IU/kg was also performed once a day in each group of STZ-diabetic rats. After ten-days of treatment, all rats challenged with exogenous insulin. According to the method described previously (Wu et al., 2002), an intravenous insulin challenge test was performed by giving 0.05–2.5 IU/kg of short-acting human insulin to these STZ-diabetic rats. Blood samples (0.2 ml) from the femoral vein were drawn after 30 min following the intravenous insulin challenge test, for the measurement of the plasma glucose concentrations. At the end of the repeated treatment, not only the body weight but also the daily amount of food intake or water intake in Hon-Chi-treated (150 mg/kg) STZ-diabetic rats was measured, to compare with the rats treated with vehicle. STZ-diabetic rats receiving an oral administration of metformin at 320 mg/kg, the effective dose used in Zucker rats (Rouru, Huupponen, Pesonen, & Koulu, 1992), were used as the positive control. The rats received oral administration of metformin at 320 mg/kg, three times daily for 15-days, (the effective duration for oral administration of metformin (Rouru et al., 1992)), or the same volume of vehicle, to carry out the intravenous insulin challenge test in the same way. The difference between the two groups in response to exogenous insulin was also compared, to measure the plasma glucose lowering activity (%).

2.7. Statistical analysis

Data are expressed as the mean ± SE for each group of animals. Statistical differences among groups were determined by using two-way ANOVA. The Dunnett range *post-thoc* comparisons were used to determine the source of significant differences where appropriate. A P-value < 0.05 was considered statistically significant.

3. Results and discussion

In insulin resistance, both *in vivo* and *in vitro*, certain intracellular signalling pathways are more resistant to the stimulation of insulin than others (Kruszynska & Olefsky, 1996). The action of insulin is important in body adaptation to the ingestion of nutrients, in particular, dietary carbohydrates (Bessesen, 2001). Insulin resistance in fructose-rich chow-fed rat is documented to be similar to that in genetically obese Zucker rats, since high intake of fructose in rats has been demonstrated to produce a decline of insulin sensitivity in peripheral tissues associated with insulin resistance (Liu et al., 2005). Due to its recent increase in fructose consumption, the fructose-rich chow-fed rat seems suitable to investigate. The increased consumption of dietary fructose might be one of the factors responsible for the development of obesity and the accompanying insulin resistance syndrome.

The plasma glucose levels in fructose-rich chow-fed rats receiving an oral administration of Hon-Chi were determined. As shown in Fig. 1, the fasting plasma glucose levels were significantly increased in the fructose-rich chow fed-rats, as compared with those in standard chow-fed groups. A dose-dependent decrease of the plasma glucose level in the fructose-rich chow fed-rats, which received an oral administration of Hon-Chi from 25 mg/kg to 150

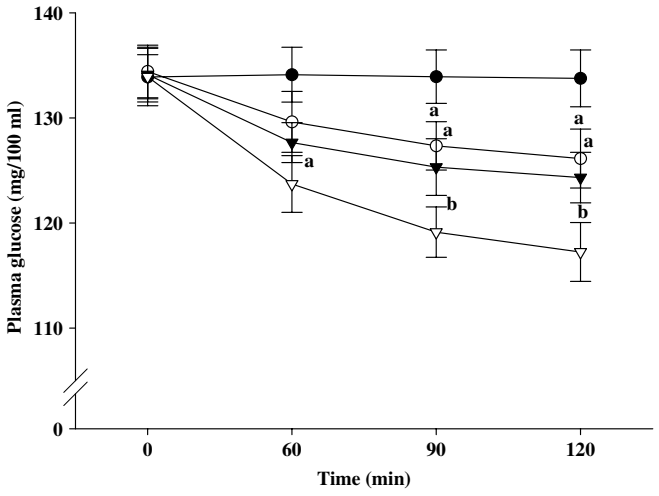

Fig. 1. Changes of plasma glucose levels in fructose-rich chow fed-rats treated with Hon-Chi at 25 mg/kg (○), 50 mg/kg (▲) and 150 mg/kg (Δ); vehicle control (●). Values (mean ± SE) were obtained from each group of eight animals. [a]$P < 0.05$ and [b]$P < 0.01$ versus data from control at the corresponding time.

mg/kg was obtained. The effect of Hon-Chi reached a plateau within 90 min and was maintained for 120 min or more. After 90 min, Hon-Chi at 150 mg/kg significantly decreased the plasma glucose of fructose-rich chow fed-rats from 143.8 ± 2.2 mg/100 ml to 121.2 ± 1.7 mg/100 ml, showing a lowering activity of $15.6 \pm 2.1\%$. After 120 min, Hon-Chi at 150 mg/kg resulted in a plasma glucose lowering activity of $16.1 \pm 3.1\%$, which was not significantly higher. Thus, the effect of Hon-Chi was determined using blood samples collected after 90 min in subsequent experiments.

The plasma insulin levels in fructose-rich chow-fed rats receiving an oral administration of Hon-Chi were also investigated. The plasma insulin levels of the rats, which received four-weeks fructose feeding were significantly raised, compared with the standard chow-fed group (183.6 ± 6.5 μU/ml and 19.8 ± 5.2 μU/ml, respectively; $P < 0.01$). After receiving an oral treatment with Hon-Chi for 90 min, the plasma insulin concentrations in fructose-rich chow fed-rats slightly decreased to 178.3 ± 7.14 μU/ml, although this effect was not significantly ($P > 0.05$) different from the fructose-fed rats which received saline. These results implied that Hon-Chi seems beneficial in the improvement of insulin action for lowering plasma glucose.

Clinically, the initial therapy for type 2 diabetes has been shifting from secretagogues and alpha-glucosidase inhibitors to agents for improvement of insulin sensitivity, known as insulin sensitizers, including thiazolidinediones and others (De Vos et al., 1996; Kecskemeti et al., 2002). Several methods have been established to assess insulin sensitivity in animals and humans. The hyperinsulinaemic-euglycaemic glucose clamp is one of the most widely employed methods but this technique is experimentally demanding and costly (Bessesen, 2001). In recent studies, glucose or insulin area under the curve (AUC) after glucose loading du ring the whole test or du ring specific intervals has seen widespread use as a simple method (Bessesen, 2001; Liu et al., 2005). The glucose-insulin index, defined as the product of the glucose and insulin AUCs, is an index of *in vivo* peripheral insulin action showing insulin sensitivity. We employed the glucose-insulin index during the IPGTT, to clarify if Hon-Chi had the capability to enhance glucose disposal in fructose-rich chow-fed rat.

Four-weeks after fructose feeding, the body weight of rats was significantly raised, compared with the standard chow-fed group (Table 1). Also, the daily food intake and the daily water intake in fructose-rich chow-fed rats were higher than those of standard chow-fed group (Table 1). No significant differences were observed in the body weights among fructose-fed groups with or without Hon-Chi treatment at the corresponding time points. Moreover, Hon-Chi treatment for three-days did not cause any change in daily food intake nor daily water intake in fructose-rich chow-fed rats (Table 1).

The effect of a three-day treatment of Hon-Chi on the changes of glucose disposal rate in fructose-rich chow-fed rats was further determined. Increases of plasma levels in glucose and insulin during IPGTT in the fructose-rich chow-fed rats were markedly higher than those in the standard chow-fed group (Fig. 2a and b). Also, the AUC levels of plasma glucose during IPGTT in fructose-rich chow-fed rats were significantly elevated to near 1.6-fold of the standard chow-fed animals (Fig. 2c). The AUC levels of the plasma insulin during IPGTT in the fructose-rich chow-fed rats were also significantly higher than those in the standard chow-fed group (Fig. 2c). Moreover, the glucose-insulin index in fructose-rich chow-fed rats receiving an oral glucose load was raised to 13-fold of that obtained from the standard chow-fed group (Fig. 2d). Repeated treatments with Hon-Chi in fructose-rich chow-fed rats for three-days caused a dose-dependent reduction of plasma glucose during IPGTT (Fig. 2a). The total AUC for the glucose response in the fructose-rich chow-fed rats treated with Hon-Chi (150 mg/kg) for three-days was lower by about 70% of that from their vehicle-treated counterparts during IPGTT (Fig. 2c). Also, insulin values in plasma from the fructose-rich chow-fed rats and the incremental area under the insulin curve during IPGTT were lowered by 150 mg/kg Hon-Chi to near 70% of the values obtained from the vehicle-treated group (Fig. 2b and c). Moreover, Hon-Chi (150 mg/kg) effectively decreased the value of the glucose-insulin index in fructose-rich chow-fed rats during IPGTT to about 60% of the values obtained from vehicle-treated animals (Fig. 2d). This shows that

Table 1
General characteristics of fructose-rich chow-fed rats after oral treatment of Hon-Chi at indicated dose, three times daily for three-days

Group	Body weight (g/rat)	Food intake (g/rat)	Water intake (ml/rat)	Plasma glucose (mg/100 ml)	Plasma insulin (μU/ml)
Standard chow-fed					
Vehicle	212.6 ± 6.2^{b}	18.7 ± 5.6^{b}	30.7 ± 7.8^{b}	90.6 ± 5.8^{a}	20.6 ± 5.7^{b}
Fructose-rich chow-fed					
Vehicle	350.6 ± 7.2	43.2 ± 6.1	68.7 ± 6.4	146.2 ± 4.8	185.3 ± 6.8
Hon-Chi (25 mg/kg)	346.4 ± 9.2	42.6 ± 6.7	70.2 ± 8.1	139.3 ± 5.2	175.6 ± 7.1
Hon-Chi (50 mg/kg)	340.2 ± 8.4	39.4 ± 7.3	71.2 ± 9.2	128.2 ± 4.4^{a}	160.4 ± 8.3
Hon-Chi (150 mg/kg)	348.2 ± 6.9	41.6 ± 6.4	69.8 ± 7.8	118.4 ± 5.9^{b}	148.2 ± 7.4^{a}

Data are means ± SE. Assays were performed on tissue from eight different animals in each group. $^{a}P < 0.05$ and $^{b}P < 0.01$ compared to the values of fructose-rich chow-fed rats treated with vehicle, respectively.

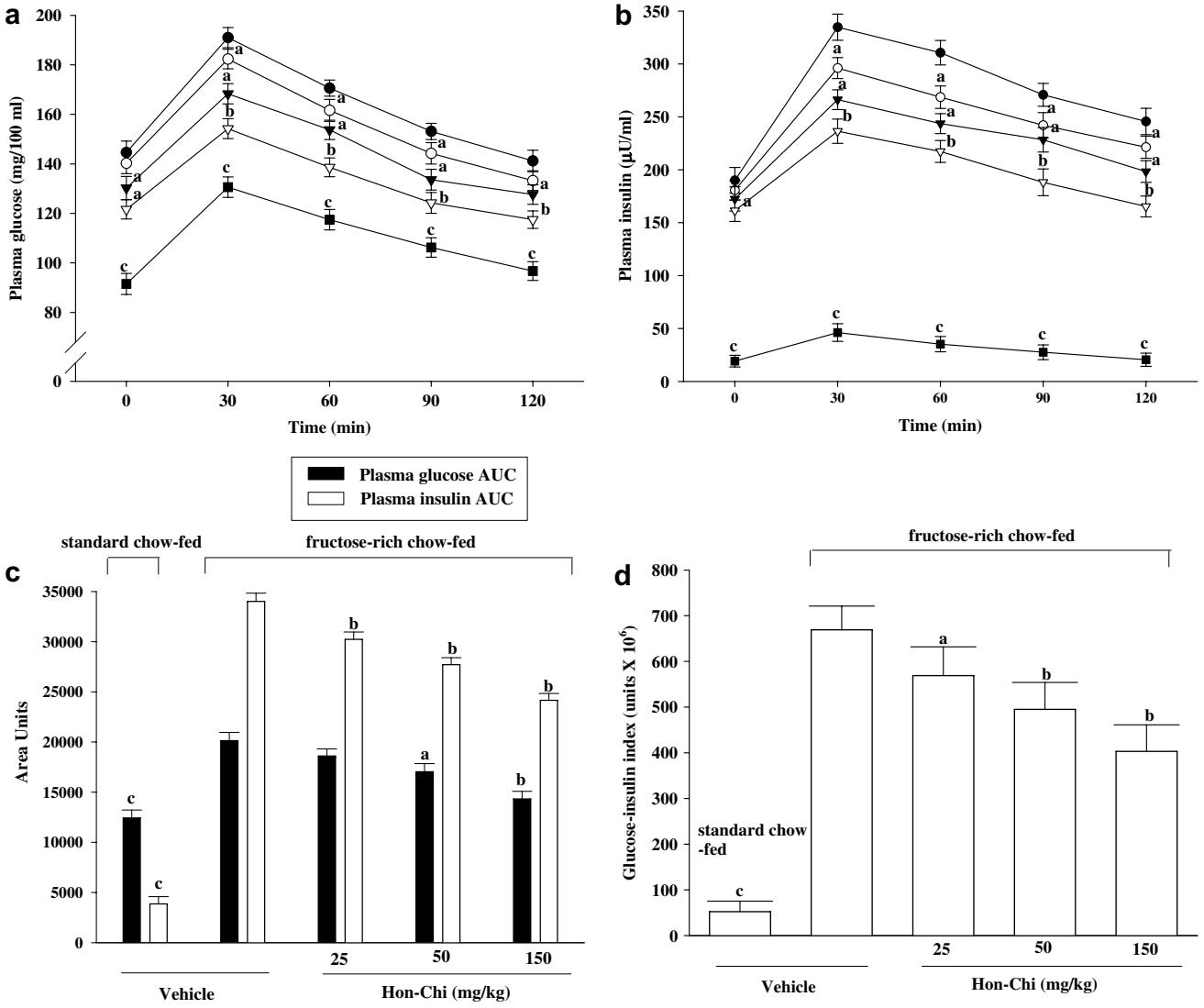

Fig. 2. (a) Plasma glucose responses during the intraperitoneal glucose (1 g/kg) tolerance test (IPGTT) in fructose-rich chow fed-rats treated with or without Hon-Chi, (b) plasma insulin responses during IPGTT in these rats. Fructose-rich chow fed-rats treated with Hon-Chi at the dosage of 25 mg/kg (○), 50 mg/kg (▲) and 150 mg/kg (Δ), three times daily, for three-days. Standard chow fed-rats (■) and fructose-rich chow fed-rats (●) not fed Hon-Chi, (c) the incremental areas under the curves (AUC) for levels of plasma glucose and plasma insulin in rats during IPGTT and (d) the glucose-insulin index calculated as the product of the plasma glucose AUC and plasma insulin AUC for each animal. Values (mean ± SE) were obtained from each group of eight animals. $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$ versus data from fructose-rich chow fed-rats treated with vehicle at the corresponding time, respectively.

Hon-Chi has an ability to correct the impairment of insulin-stimulated glucose disposal in rats with insulin resistance. The action of endogenous insulin can be increased by Hon-Chi, to lower the insulin resistance induced by fructose-rich chow. Hon-Chi has been used in China for a long time to enhance the colour and flavour of food, as well as a traditional medication for digestive and vascular disorders, without evidence of adverse effect or toxicity (Journoud & Jones, 2004). Therefore, Hon-Chi seems useful as an adjuvant for management of insulin resistance.

Intervention prior to the onset of type 2 diabetes may be the only way of preventing its complications (Younis et al., 2004). Whether Hon-Chi has the capability to prevent or delay the progression to type 2 diabetes was then investigated. Four-weeks after fructose feeding, the body weight of rats was significantly raised, compared with the standard chow-fed group (342.6 ± 7.1 g/rat *vs.* 208.6 ± 6.4 g/rat, $P < 0.05$). However, the body weight of those rats which received the oral treatment of Hon-Chi (150 mg/kg) during the fructose-rich chow-feeding period for 28-days was reduced to 274.6 ± 7.4 g/rat. The daily food intake and the daily water intake in rats treated with vehicle during the fructose-rich chow-feeding period were elevated to 45.2 ± 5.7 g/rat and 64.8 ± 4.9 ml/rat, respectively. Treatment with Hon-Chi (150 mg/kg) accompanying the fructose-rich chow-feeding for 28-days reduced the daily food intake of these rats to 32.4 ± 4.2 g/rat and reduced the daily water intake to 53.7 ± 5.1 ml/rat. In addition, fasting plasma glucose concentrations were markedly higher in the fructose-fed rats than in the standard chow-fed controls

(147.2 ± 4.7 mg/100 ml *vs.* 89.3 ± 5.3 mg/100 ml, $P < 0.05$). The fasting plasma insulin concentration in the fructose-fed rats was also markedly increased, compared with that of standard chow fed controls (179.8 ± 6.7 μU/ml *vs.* 18.3 ± 3.7 μU/ml; $P < 0.01$). In the presence of Hon-Chi (150 mg/kg), the plasma levels of glucose and insulin in rats fed with fructose-rich chow at 28th day were significantly ($P < 0.05$) decreased to 121.3 ± 5.9 mg/100 ml and 154.3 ± 6.2 μU/ml, respectively.

The plasma glucose lowering activity of tolbutamide is believed to depend on the secretion of endogenous insulin (Chang, Lin, Chi, Liu, & Cheng, 1999; Weinkove, Weinkove, & Pimstone, 1976). Loss of the plasma glucose lowering response to tolbutamide has been interpreted as the development of insulin resistance (Chang et al., 1999; Weinkove et al., 1976). The plasma glucose lowering activity of tolbutamide was employed as an indicator to evaluate the induction of insulin resistance in rats. Thus, effects of Hon-Chi on the induction of insulin resistance in rats fed with fructose-rich chow were further determined by the plasma glucose lowering activity of tolbutamide. In the presence of tolbutamide (10 mg/kg), the plasma glucose concentration in rats before fructose-rich chow feeding was reduced from 94.2 ± 3.1 mg/100 ml to 63.2 ± 2.7 mg/100 ml; the plasma glucose lowering activity of tolbutamide was about 33.2 ± 3.9% (Table 2). After three-day feeding of high fructose chow, the plasma glucose concentration in rats, which had received the tolbutamide injection, changed from 109.4 ± 4.2 mg/100 ml to 77.6 ± 3.1 mg/100 ml; the plasma glucose lowering activity of tolbutamide became 29.1 ± 3.6% (Table 2). After fructose-rich chow feeding for 15-days, the plasma glucose concentration in rats was elevated to 127.8 ± 3.2 mg/100 ml; tolbutamide lowered the plasma glucose concentration of these rats to 111.9 ± 2.6 mg/100 ml. The plasma glucose concentration in rats fed with fructose-rich chow at 28th day was markedly increased to 145.2 ± 3.4 mg/100 ml and it was only decreased to 137.2 ± 2.8 mg/100 ml by the same treatment of tolbutamide. In fact, the plasma glucose lowering activity of tolbutamide in rats was reduced from 12.4 ± 2.1% after fructose-rich chow feeding for 15-days to 5.4 ± 1.8% in rats fed with fructose-rich chow at the 28th day. However, the plasma glucose concentration in rats fed with standard chow at the 28th day and receiving tolbutamide treatment was changed from 93.6 ± 4.4 mg/100 ml to 61.7 ± 5.3 mg/100 ml; the plasma glucose lowering activity of tolbutamide was still about 34.1 ± 4.5%.

In rats receiving oral treatment with Hon-Chi (150 mg/kg) during fructose-rich chow feeding for three-days, the plasma glucose concentration in response to tolbutamide was reduced from 104.3 ± 4.1 mg/100 ml to 72.4 ± 3.5 mg/100 ml; the plasma glucose lowering activity of tolbutamide was still 30.7 ± 3.2% (Table 2). Also, tolbutamide lowered the plasma glucose concentration from 118.2 ± 3.7 mg/100 ml to 92.7 ± 2.9 mg/100 ml in fructose-rich chow feeding rats receiving Hon-Chi (150 mg/kg) treatment for 15-days; the plasma glucose lowering activity of tolbutamide was 21.6 ± 3.3% (Table 2). At the 28th day of fructose-rich chow feeding, tolbutamide lowered the plasma glucose concentration from 128.5 ± 3.6 mg/100 ml to 112.9 ± 2.8 mg/100 ml in rats receiving Hon-Chi at 150 mg/kg; the plasma glucose lowering activity of tolbutamide remained 12.1 ± 3.7% (Table 2). Thus, the decrease of response to tolbutamide by fructose-rich chow in rats was markedly reversed by the treatment of Hon-Chi, showing that Hon-Chi has an ability to delay the development of insulin resistance in rats. The possible mechanism is related to the change in insulin sensitivity. Thus, we investigated the effect of Hon-Chi on insulin sensitivity.

The previous method developed to evaluate insulin sensitivity *in vivo* was the intravenous insulin challenge test based on the change of plasma glucose level after a bolus injection of regular insulin (Wu et al., 2002). Thus, we employed this insulin challenge test in STZ-diabetic rats. The advantage of STZ-diabetic rats used in the present study is the negligible effect of endogenous insulin. Plasma glucose lowering action is directly due to the action of exogenous insulin in the STZ-diabetic rat. The obtained results can thus be used to indicate insulin sensitivity. The basal plasma glucose concentration in STZ-diabetic rats was 424.3 ± 5.7 mg/100 ml. The plasma glucose lowering activity of short-acting human insulin (exogenous insulin), at doses from 0.05 to 2.5 IU/kg in STZ-diabetic rats receiving Hon-Chi (150 mg/kg, three times daily) for 15-days, was markedly higher than that in the control

Table 2
Effect of tolbutamide on plasma glucose in rats receiving oral administration of Hon-Chi or vehicle during the fructose-rich chow feeding period

Days	Plasma glucose (mg/100 ml)					
	Standard chow-fed rats		Fructose-rich chow-fed rats			
	Vehicle-treated		Vehicle-treated		Hon-Chi (150 mg/kg)-treated	
	Vehicle	Tolbutamide (10 mg/kg, IP)	Vehicle	Tolbutamide (10 mg/kg, IP)	Vehicle	Tolbutamide (10 mg/kg, IP)
0	91.6 ± 4.2	61.4 ± 3.2	94.2 ± 3.1	63.2 ± 2.7	93.6 ± 5.3	62.7 ± 3.8
3	91.9 ± 4.7	61.6 ± 3.8	109.4 ± 4.2[a]	77.6 ± 3.1	104.3 ± 4.1	72.4 ± 3.5
15	92.4 ± 3.8	60.2 ± 4.1	127.8 ± 3.2[a]	111.9 ± 2.6[a]	118.2 ± 3.7[a]	92.7 ± 2.9[a]
28	93.6 ± 4.4	61.7 ± 5.3	145.2 ± 3.4[b]	137.2 ± 2.8[b]	128.5 ± 3.6[a]	112.9 ± 2.8[a]

Vehicle indicated the same volume of saline to dissolve Hon-Chi or tolbutamide. Values (mean ± SE) were obtained from eight experiments. [a] $P < 0.05$ and [b] $P < 0.01$ versus data from rats at day 0, respectively.

group receiving the same volume of vehicle (Fig. 3). The plasma glucose lowering activity of exogenous insulin in Hon-Chi-treated group was about 42.2 ± 4.8% at 1 IU/kg and rose to 50.2 ± 5.1% at a dose of 2.5 IU/kg (3). Actually, intravenous challenge with exogenous insulin at the dose of 1 IU/kg resulted in a plasma glucose lowering activity of 46.2 ± 4.6% incresing to 53.3 ± 4.9% at the dose of 2.5 IU/kg in STZ-diabetic rats receiving a 15-day metformin (320 mg/kg, three times daily) treatment (3). We found that the ability of Hon-Chi to improve insulin sensitivity was similar to that of metformin, because the improvement of insulin sensitivity by Hon-Chi after 15-days treatment in STZ-diabetic rats was similar to the activity of metformin. Therefore, it can be considered that Hon-Chi has a metformin-like action that enhances insulin sensitivity *in vivo*.

The plasma glucose level in STZ-diabetic rats treated with exogenous insulin (2.5 IU/kg) and Hon-Chi (150 mg/kg) at the 15th day was markedly ($P < 0.01$) reduced to 268.4 ± 5.4 mg/100 ml. The initial plasma insulin level in STZ-diabetic rats was only 0.3 ± 0.2 μU/ml ($n = 8$). After 15-days of 150 mg/kg Hon-Chi treatment, the plasma insulin level in STZ-diabetic rats remained 0.4 ± 0.2 μU/ml ($n = 8$), similar to the value before treatment. Both the daily food and water intakes in STZ-diabetic rats treated with Hon-Chi at the 15th day were reduced to 40.3 ± 6.2 g/rat and 60.2 ± 7.3 ml/rat, respectively ($n = 8$). However, the daily food intake (55.4 ± 5.7 g/rat) and water intake (78.2 ± 5.4 ml/rat) was still higher in STZ-diabetic rats receiving similar treatment of vehicle at the same period ($n = 8$). Also, the body weight in STZ-diabetic rats receiving the treatment of Hon-Chi for 15-days was elevated to 193.2 ± 6.1 g/rat, compared with that of the vehicle-treated control (174.6 ± 5.2 g/rat, $n = 8$).

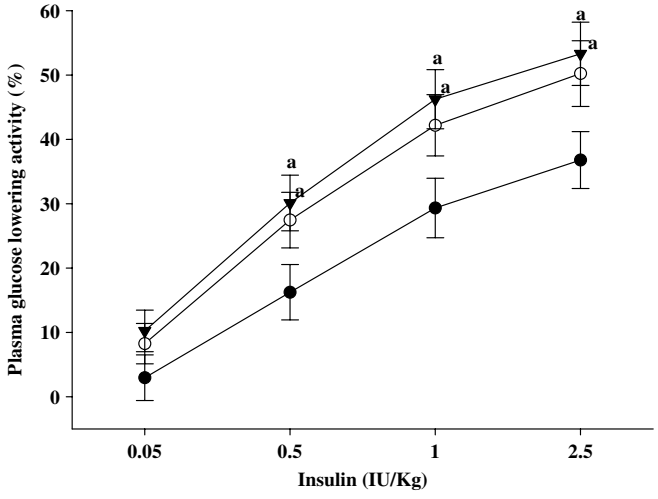

Fig. 3. Change of intravenous insulin challenge test in STZ-diabetic rats treated with an oral administration of Hon-Chi at 150 mg/kg (○) or metformin at 320 mg/kg (▲) for 15-days, compared to a control receiving similar treatment with vehicle (●). Values (mean ± SE) were obtained from each group of eight animals. [a]$P < 0.05$ and [b]$P < 0.01$ versus data from STZ-diabetic rats treated with vehicle (closed circles), respectively.

In an insulin resistant state, defects in the insulin signal cascade leading to impaired glucose utilisation have been proposed as a key cause for the pathogenesis of this disorder (Ogawa, 2001). It has been indicated that metformin improves the insulin sensitivity in insulin resistant subjects by activating post-receptor insulin signalling pathways (Hundal & Inzucchi, 2003). Due to the lowering of glucose level without increase of insulin secretion, metformin has been considered an insulin sensitiser (Hundal & Inzucchi, 2003). Metformin has many beneficial effects in adults with type 2 diabetes, including weight reduction, decreased hyperinsulinaemia, improved lipid profiles, augmented fibrinolysis and enhanced endothelial function (Hundal & Inzucchi, 2003). In fact, insulin sensitisers have the ability to improve insulin sensitivity *via* many mechanisms, such as a direct effect on muscle insulin sensitivity, the stimulation of insulin-sensitive fat cells, or the regulation of leptin expression (De Vos et al., 1996). Thus, more studies are required to identify if Hon-Chi might improve insulin resistance, through the modulation of the insulin signaling pathway to reverse the responsiveness of insulin.

Preparation of Hon-Chi following ancient methods by fermenting the fungal strain *Monascus* strains on moist and sterile rice indicated the presence of a group of metabolites belonging to the monacolin family of polyketides, reported to exhibit a cholesterol-lowering action, by inhibiting the HMG-CoA reductase (Heber et al., 1999; Journoud & Jones, 2004). However, it has been suggested that the hypolipidaemic effect of Hon-Chi cannot be accounted for only by the inhibition of HMG-CoA reductase, but by a synergy of all components (Heber et al., 1999; Ma et al., 2000). In fact, several publications have reported the presence of other components in red yeast rice, such as the plant sterols β-sitosterol, campesterol, stigmasterol, saponin and sapogenin; isoflavones and isoflavone glycosides; selenium, and zinc (Heber et al., 1999; Ma et al., 2000). It has been documented that treatment with aqueous extract of *M. purpureus* suppressed fructose-induced elevation in total cholesterol levels and enhanced the recovery of high-density lipoprotein cholesterol but did not alter the insulin sensitivity in fructose-fed and control rats (Hsieh & Tai, 2003). Thus, the active principle(s) contained in Hon-Chi for the improvement of insulin resistance need further investigation. Nevertheless, Hon-Chi can be used as a food-based adjuvant therapy for diabetic patients with insulin resistance or for individuals who have an urgent need to prevent insulin resistance and/or impaired glucose metabolism (Younis et al., 2004).

In conclusion, the present study found that Hon-Chi has the ability to improve insulin resistance in rats induced by fructose-rich chow. The obtained data strengthen the basis for recommending Hon-Chi as a candidate to improve insulin sensitivity in the future.

Acknowledgements

We thank Standard Chem. & Pharm. Co., Ltd. (Hsin-Ying, Taiwan) for the kind supply of Hon-Chi. Thanks are also due to Professor Y.C. Tong for editing and Miss Y.P. Lin for technical assistance.

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