DOI:10.1021/jf8028539

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Baicalin Improves Antioxidant Status of Streptozotocin-Induced Diabetic Wistar Rats

Viduranga Y. Waisundara,[†] Annie Hsu,[‡] Benny Kwong-Huat Tan,[‡] and Dejian Huang*,[†]

[†]Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, and [‡]Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, 10 Medical Drive, Singapore 117543

This study investigated the antioxidant and antidiabetic effects of baicalin, as well as its effects in combination with the antidiabetic drug metformin. Three groups of streptozotocin-induced diabetic rats were given the following treatments for 30 days: (1) 500 mg/kg metformin; (2) 120 mg/kg baicalin; (3) 500 mg/kg metformin + 120 mg/kg baicalin. In addition, vehicle-treated diabetic and nondiabetic controls were used in the experiment. The rats treated with baicalin and metformin + baicalin had significantly elevated ($p < 0.05$) hepatic activities of superoxide dismutase, catalase, and glutathione peroxidase compared with the vehicle- and metformin-treated groups. Plasma and hepatic lipid peroxide concentrations of the baicalin- and baicalin + metformin-treated groups were significantly reduced ($p < 0.05$). In addition, baicalin significantly reduced plasma and hepatic triglycerides and cholesterol levels. The study thus showed that baicalin mitigated oxidative stress as well as enhanced the antidiabetic effect of metformin by improving the antioxidant status.

KEYWORDS: Baicalin; diabetes; metformin; oxidative stress; streptozotocin

INTRODUCTION

Recent evidence suggests that oxidative stress contributes to the pathogenesis of type 1 and type 2 diabetes and its complications (*1*). Diabetic hyperglycemia is known to cause a variety of pathologic changes in small vessels, arteries, and peripheral nerves resulting in hyperglycemia-induced complications (*2*−*4*). Activation of protein kinase C isoforms, increased formation of glucose-derived advanced glycation end (AGE)-products, and increased glucose flux through the aldose reductase pathway have been recently shown to be consequences of a single common mechanism, hyperglycemia-induced mitochondrial superoxide overproduction (*5*, *6*). Oxidative stress initiated through these pathways is commonly measured through biomarkers produced through ROS activity, such as lipid peroxides. One major complication of hyperglycemia is disorder in macro- and microvascular and lipid peroxidation on LDL may play a critical role and lipid oxidative stress marker is pertinent to assess the risk of diabetes complications. It is a well-known and practiced theory that mitigating oxidative stress by antioxidants may prevent diabetic complications (*7*−*10*).

Plant flavonoids exert a wide range of biochemical and pharmacological activities including antioxidant, vasodilatory, anti-inflammatory, antibacterial, immune-stimulating, antiallergic, and antiviral effects (*11*−*14*). Our previous in vivo study on the ethanolic extract of *Scutellaria baicalensis*—an herb having abundant flavonoid compounds—showed significant antioxidant and antidiabetic effects (*15*). Analysis of this herbal extract revealed the presence of baicalin, baicalein, and wogonin as the three major flavonoid compounds. However, baicalin (**Figure 1**)—a flavone glycoside (baicalein-7-glucuronide)—was the major compound found in the freeze-dried ethanolic extract of *S. baicalensis*. The extract contained 29.6% of baicalin as compared with 1.3 and 0.7% of baicalein and wogonin, respectively. Thus, this study in streptozotocin (STZ)-induced diabetic Wistar rats was carried out to highlight baicalin as the primary bioactive compound in the freeze-dried ethanolic herbal extract of *S. baicalensis*. In addition, the antioxidant and antidiabetic activity of baicalin in STZ-induced diabetic Wistar rats has not been investigated to date. With reference to our previous study on *S. baicalensis*, the effects of the flavonoid in combination with metformin—a commonly used oral hypoglycemic prescription medicine—were also investigated. Metformin primarily increases insulin-receptor site density on erythrocytes to result in increased glucose uptake in muscles (*15*, *16*). Although epidemiological studies have shown a comparatively lower incidence of side effects associated with metformin, no reduction in diabetic oxidative stress levels has been reported. As with the previous study, it was our aim to demonstrate whether the addition of baicalin to metformin could potentially increase the antioxidant defenses as well as enhance the antidiabetic effects of metformin in the rat with experimental diabetes.

MATERIALS AND METHODS

Chemicals. Metformin and baicalin were purchased from Sigma Chemical Co. (St Louis, MO). The purity of baicalin was verified in-house using high-performance liquid chromatography (HPLC). All other chemicals used were of analytical grade and purchased from either

*Corresponding author [telephone (65)-6516-8821; fax (65)-6775-7895; e-mail chmhdj@nus.edu.sg].

 Published on Web 4/13/2009

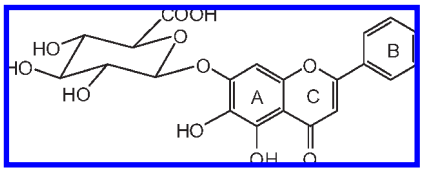

Figure 1. Chemical structure of baicalin (baicalein-7-glucuronide). The *o*-hydroxyl groups on the A-ring as well as the ketone group at the C4 position enable this compound to be a radical scavenger and metal chelator.

Sigma Chemical Co. or BDH Chemicals (BDH Ltd., Poole, U.K.), unless otherwise specified.

Antioxidant Activity Measurement. Chemically, the peroxyl radical scavenging activity of baicalin was analyzed using a high-throughput oxygen radical scavenging capacity assay as described by Huang et al. (*17*). Baicalin was reconstituted in distilled water to a concentration of 0.1 mg/mL before final analysis. The in vivo antioxidant activity was studied using rats as approved by the IACUC (Institutional Animal Care and Use Committee). Locally bred male Wistar rats weighing 250−300 g each were obtained from the Laboratory Animal Center, National University of Singapore, and housed in a room with a controlled temperature (22 ± 3 °C) on a 12:12 light/dark cycle. Unless otherwise specified, animals had free access to pelleted food containing the AIN-93 M Maintenance Purified Diet (Glen Forrest, WA, Australia) with tap water ad libitum. Diabetes was induced in the rats after fasting for 18 h by intraperitoneal injection of 65 mg/kg of body weight of streptozotocin (STZ), which was dissolved in citrate buffer (0.01 M, pH 4.5) just before use. The diabetic state was assessed by measuring the nonfasting serum glucose level 5 days after the administration of STZ. Rats with a serum glucose level above 300 mg/dL as well as polydipsia, polyuria, and polyphagia were selected for the experiment. The diabetic rats were divided into four groups (six rats each group) with the following treatments: (1) M, 500 mg/kg metformin; (2) B, 120 mg/kg baicalin; (3) MB, 500 mg/kg metformin plus 120 mg/kg baicalin; (4) DC, diabetic control. A nondiabetic control (NDC) was also used in the experiment. The dosage of baicalin administered to the rats was calculated with reference to our previous in vivo study on *S. baicalensis* and the percentage of baicalin contained in the freeze-dried extract of this herb (*15*). To make a fair comparison, the dosage of metformin remained unchanged as per our previous study as well. The respective treatments were orally administered twice daily for 30 days. The body weight and food and water intakes of the rats were monitored on a daily basis. Blood was collected from the tail vein of the rats for monitoring of weekly blood glucose levels, total cholesterol (TC), triglycerides (TG), and plasma lipid peroxide concentrations. On the evening of day 30, the rats were fasted overnight and euthanized the following morning by decapitation. Blood was collected by drainage from the carotids following euthanasia and kept in 200 μL aliquots at −80 °C until time for assay. The liver, pancreas, and kidney of the rats were removed, sectioned into 0.5 g aliquots, immersed in liquid nitrogen, and stored at −80 °C until time for assay.

Catalase (CAT), Superoxide Dismutase (SOD), and Glutathione Peroxidase (GPx). Liver samples (0.50 g) were homogenized in potassium phosphate buffer (5.0 mL, 0.05 M with 0.1 mM EDTA, pH 7.8). The homogenate was centrifuged at 12000*g* for 10 min at 4 °C. The supernatant was divided into aliquots and kept at −80 °C for the enzyme assays. CAT, SOD, and GPx activities were determined using the respective assay kits from Cayman Chemical Co. (An arbor, MI). Protein expression was analyzed by standard Western blot using the Laemmli SDS-PAGE for separation of proteins. Primary antibodies for CAT, SOD, and GPx were purchased from Santa Cruz Biotechnology Inc., (Santa Cruz, CA). Primary antibody binding was visualized by horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechmology Inc.) and an enhanced chemiluminescence (ECL) assay kit (Pierce Biotechnology, IL).

Plasma, hepatic, kidney, and pancreatic lipid peroxides were quantified according to the method by Hay et al. (*18*). Details of the methodology are described in the Supporting Information. Plasma and pancreatic insulin concentrations were determined by enzyme-linked immunosorbent assay (ELISA) using assay kits provided by Crystal Chemical Inc. (Santa Monica, CA). Prior to analysis, the pancreatic tissues (0.10 g) were disintegrated by ultrasound in 5 mL of acid−alcohol solution (75% ethanol, 1.5% 12 M HCl, and 23.5% distilled water) for 1 min at 4 °C. The homogenates were kept overnight at −20 °C and centrifuged at 12000*g* for 15 min. Plasma glucose concentrations were measured using the enzymatic glucose reagent (Thermo Electron Corp., Victoria, Australia) according to the glucose oxidase method. Plasma TC and TG concentrations were measured by the colorimetric method using diagnostic kits from Infinity (Thermo Electron Corp.). Insulin and leptin concentrations were determined by ELISA using assay kits purchased from Crystal Chemical Inc.

Determination of Hepatic TG, TC, and Lipase Activities. Hepatic TG, TC, and lipase activities were measured at the end of the study. For hepatic TG and TC analysis, the liver samples (0.50 g) were homogenized with a Polytron homogenizer in isopropanol (10% w/v) at 4 °C for 1 min. The homogenate was centrifuged at 12000*g* for 15 min at 4 °C. The supernatants were used to assess the TC and TG contents using the reagent assay kits by Thermo Electron Corp. For assessment of hepatic lipase activity, liver samples (0.50 g) were homogenized in ammonium chloride solution (1.0 mL, 0.025 M) using a Polytron homogenizer at 4 °C for 1 min. The homogenate was centrifuged at 12000*g* for 15 min at 4 °C. The supernatant was freeze-dried for 3 days, and the resulting powder was reconstituted in an ammonium chloride solution (0.025 M) to a concentration of 10 mg/mL. A MarkerGene Fluorescent Lipase Assay kit (Eugene, OR) was used to quantify the hepatic lipase activity.

Other Biomarkers, Enzymes, and Hormones. Quantifications for glucose-6-phosphatase (G-6-Pase) activity, hepatic glycogen content, hepatic glycogen synthase content, and kidney 8-isoprostane $F_{2\alpha}$ (isoprostane) content were carried out using Marker Gene ELISA assay kits.

Statistical Analyses. Results are reported as mean ± standard error of the mean (SEM). Statistical differences among groups were calculated by using one-way ANOVA and two-way ANOVA when applicable, using SPSS version 12.0. Results were considered to be significant when p values generated from post hoc comparisons were <0.05. Post hoc comparisons were conducted with Tukey and Bonferroni tests.

RESULTS

Daily Food and Water Intakes. According to **Figure 2A**, groups M, B, and MB had statistically significant decreases in the daily water intake compared with the diabetic control ($p < 0.05$). The decrease in water intake indicates the reduction of polyuria, which is a typical characteristic of the diabetic condition. The daily food intakes of groups M, B, and MB were statistically significantly lower compared with the diabetic control ($p < 0.05$) (**Figure 2B**), but the intakes did not reach the levels of the nondiabetic controls at the end of the period of treatment. The reduction in food intake may be associated with the increase in the plasma leptin levels, which were measured at the end of the study, as shown in **Figure 2C**. A statistically significant increase in the plasma leptin levels was observed in groups M, B, and MB compared with the diabetic control ($p < 0.05$).

CAT, SOD, GPx, and GST Activities. An improvement in the antioxidant enzyme activities (compared with the diabetic control) was observed in groups B and MB ($p < 0.05$) at the end of the study (**Figure 3A**−**C**). Western blot analysis (**Figure 3D**,**E**) of the antioxidant enzymes also indicated a statistically significant increase in protein expression in groups B and MB compared with the diabetic control and group M ($p < 0.05$). It may be concluded that baicalin stimulated the protein expression of the antioxidant enzymes, which in turn resulted in the increase in their activity.

Lipid Peroxide Levels in Liver, Kidney, and Pancreas. The plasma lipid peroxides were measured on a weekly basis (**Figure 4A**). A significant decrease in plasma lipid peroxide levels was observed in groups M, B, and MB from week 2 onward, compared with the diabetic control ($p < 0.05$). From week 1 onward group MB was found to have an enhanced reduction of lipid peroxides ($p < 0.05$) compared with group M.

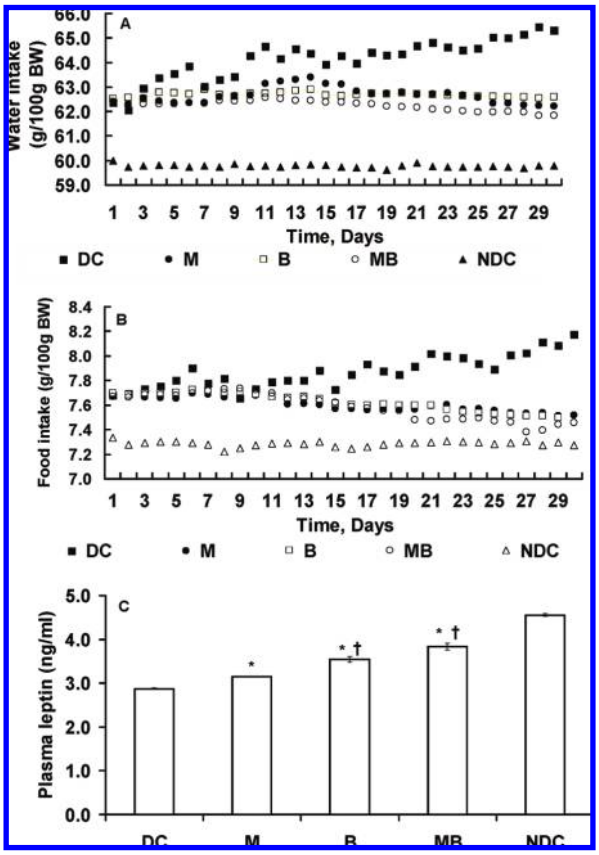

Figure 2. Effects of metformin, baicalin, and metformin + baicalin on (**A**) daily water intake, (**B**) daily food intake, and (**C**) plasma leptin content of STZ-diabetic Wistar rats. Values are expressed as mean ± standard error of the mean (SEM). Each group includes six rats per treatment. ∗, $p < 0.05$ versus the diabetic control; †, $p < 0.05$ versus the metformin-treated group.

The lipid peroxide contents of the liver, kidney, and pancreas were measured at the end of the study (**Figure 4B−D**). An enhanced reduction in the hepatic lipid peroxide was observed when the concentrations in groups M and MB were compared ($p < 0.05$). However, this decrease was not observed with kidney and pancreatic lipid peroxide contents in these two groups, although there was a statistically significant reduction in lipid peroxide contents in all of the treatment groups compared with the diabetic control ($p < 0.05$).

Plasma Glucose, Plasma, and Pancreatic Insulin Contents. The plasma blood glucose levels were measured on a weekly basis throughout the 30-day treatment. As shown in **Figure 5A**, groups M and MB showed statistically significant reductions of plasma glucose from week 1 onward as did group B from week 2 onward compared with the diabetic control ($p < 0.05$). None of the groups' plasma glucose concentrations had reached the levels of the nondiabetic control at the end of the 30-day treatment. The plasma insulin content of MB was significantly higher than that of group M ($p < 0.05$), indicating an enhancing effect of the combined treatment (**Figure 5B**). The enhancement was also observed in the pancreatic insulin content (**Figure 5C**) compared with the diabetic control.

Plasma and Hepatic TG and TC Contents and Lipase Activity. Plasma TG and TC levels were measured on a weekly basis. Significant reductions in plasma TG (**Figure 6A**) were observed in groups M, B, and MB compared with the diabetic control ($p < 0.05$). The reductions in plasma TG in groups B and MB were statistically significant compared with group M ($p < 0.05$), indicating the hypotriglyceridemic effect of the baicalin. A similar trend was observed in the plasma TC levels (**Figure 6B**). Group M did not show a significant reduction in hepatic TC; however, a significant reduction was observed in group MB compared with the diabetic control ($p < 0.05$). As shown in **Figure 6C**,**D**, hepatic TC and TG contents of group MB were also significantly increased compared with group M ($p < 0.05$). The results of the hepatic lipase activity are shown in **Figure 6E**. Groups M, B, and MB showed significant decreases ($p < 0.05$) in lipase activity compared with the diabetic control. The lipase activities in groups B and MB were significantly reduced ($p < 0.05$) compared with group M.

Glycogen Synthesis. The hepatic glycogen contents (**Figure 7A**) showed statistically significant increases in groups M, B, and MB compared with the diabetic control ($p < 0.05$), which corresponded with the glycogen synthase activities of these treatment groups (**Figure 7B**). The enhanced glycogen synthase activities may be attributed to the influence of increased hepatic glucose-6-phosphatase (G-6-Pase) activities (**Figure 7C**). Groups M, B, and MB had statistically significant increases in hepatic G-6-Pase activities compared with the diabetic control ($p < 0.05$). The influence of hepatic G-6-Pase would have resulted in a reduction of glycogenolysis and a stimulation of glycogen synthesis through glycogen synthase. Group MB had a statistically significant increase in these enzyme activities compared with group M ($p < 0.05$), indicating the enhancing effect of baicalin on glycogen synthesis.

Other Biomarkers and Hormones. The values of the other parameters—aspartate transaminase (AST) and alanine transaminase (ALT) activities, C-reactive protein (CRP), and kidney 8-isoprostane $F_{2\alpha}$ (isoprostane)—are shown in **Table 1**. Except for CRP and isoprostane, the parameters had not improved in the MB-treated group compared with group M as well as the diabetic control ($p > 0.05$). With regard to the AST and ALT values, despite the variances, the AST and ALT activities in groups DC and B were within the normal ranges of 77−157 and 24−53 U/L, respectively, which indicated that there was unlikely to be any toxicity from the treatments or possible organ damage due to the diabetic condition. The AST and ALT values of groups M and MB were above the stated normal ranges. However, histological analysis of hepatic tissues did not indicate any typical signs of toxicity-induced tissue damage, such as fibrosis of the hepatocytes (data not shown). In addition, typical in vivo conditions showing toxicity-induced tissue damage would show multiple levels of activity increases of AST and ALT, which were not observed in this study. Thus, it may be concluded that the increases in AST and ALT activities in groups M and MB were not due to the toxicity of the treatments.

DISCUSSION

Baicalin increased the antioxidant enzyme activity by increasing the expression of the proteins as observed from the Western blot data. Reductions of plasma, hepatic, kidney, and pancreatic lipid peroxides were an indirect confirmation of reduced oxidative stress levels as a result of increased antioxidant enzyme activity. Recent studies on STZ-induced diabetic Wistar rats have shown flavonoids to ameliorate diabetes-induced oxidative stress (*19*). Hyperglycemia leads to the excessive production of ROS and RNS, which in turn leads to oxidative stress products resulting in diabetic micro- and macrovascular complications (*9*, *20*, *21*). Increased expression of antioxidant enzymes would in turn result in the reduction of lipid peroxide contents in the organs. Metformin was observed to have efficiently reduced the hyperglycemic status compared with the diabetic control. Thus, as shown by previous papers, whereas metformin may not directly scavenge free radicals, the elevations in antioxidant enzymes in group M rats could be due to reduced generation of ROS resulting

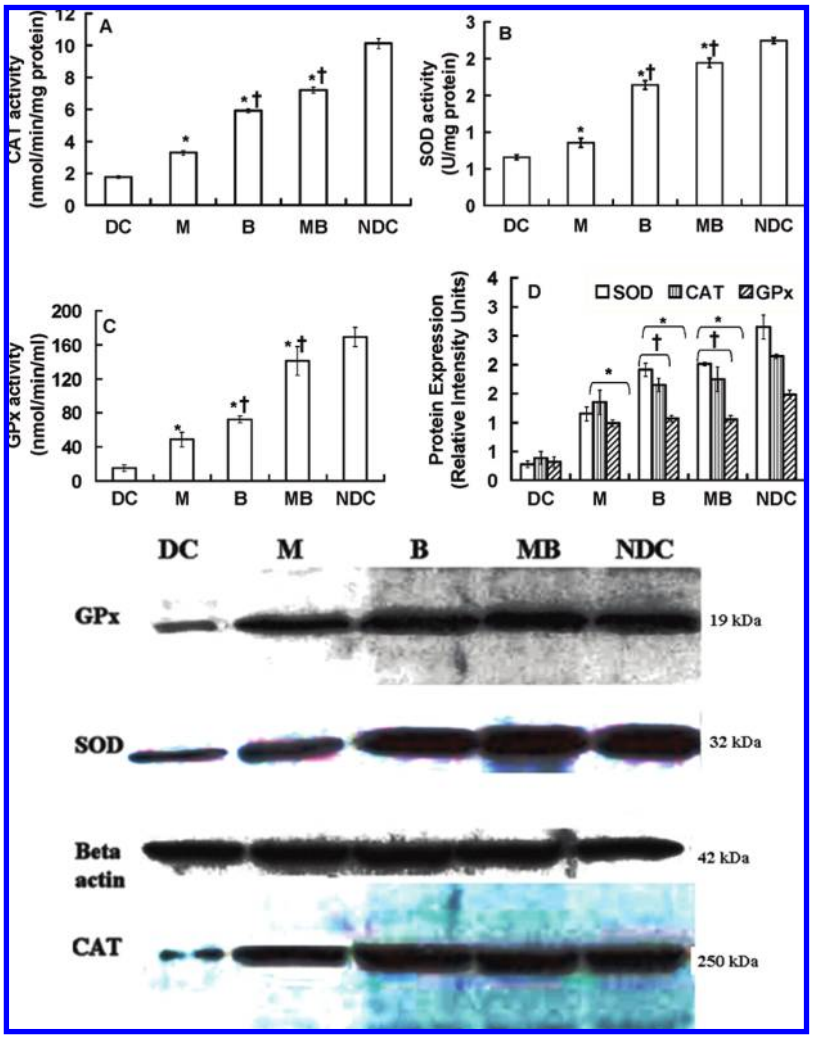

Figure 3. Effects of metformin, baicalin, and metformin + baicalin on the hepatic (**A**) catalase (CAT) activity, (**B**) superoxide disumtase (SOD) activity, (**C**) glutathione peroxidase (GPx) activity, (**D**) quantifications of the protein expressions of CAT, SOD, and GPx in STZ-diabetic Wistar rats, and (**E**) representative Western blot image of CAT, SOD, and GPx quantification. The values were measured on day 30 and are representative of 6 rats per group. Results are expressed as mean ± SEM. ∗, $p < 0.05$ versus the diabetic control; †, $p < 0.05$ versus the metformin-treated group.

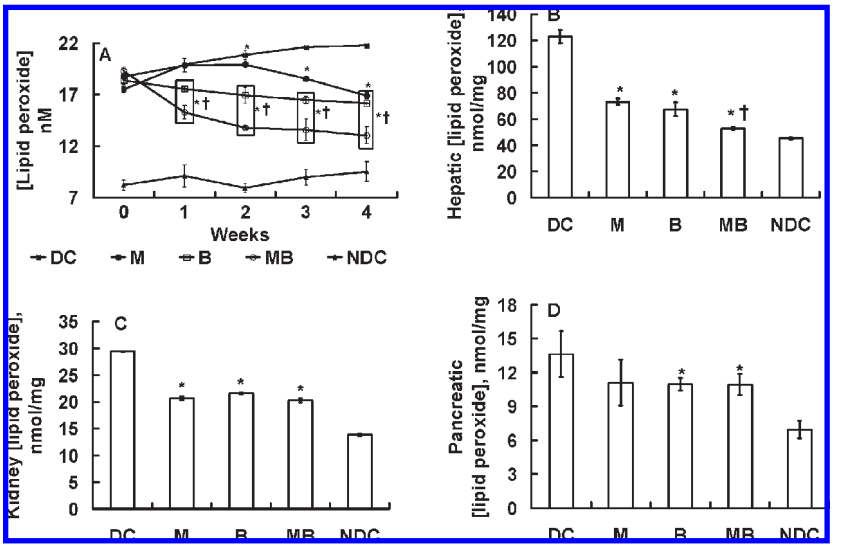

Figure 4. Effects of metformin, baicalin, and metformin + baicalin treatments of STZ-diabetic Wistar rats on (**A**) weekly plasma lipid peroxide concentrations, (**B**) hepatic lipid peroxide contents, (**C**) kidney lipid peroxide contents, and (**D**) pancreatic lipid peroxide contents. The lipid peroxide contents are expressed as mean ± SEM and represent the analysis of six rats per group. The organ lipid peroxide contents are given in terms of nM/mg wet weight of tissue sample. ∗, $p < 0.05$ versus the diabetic control; †, $p < 0.05$ versus the metformin-treated group.

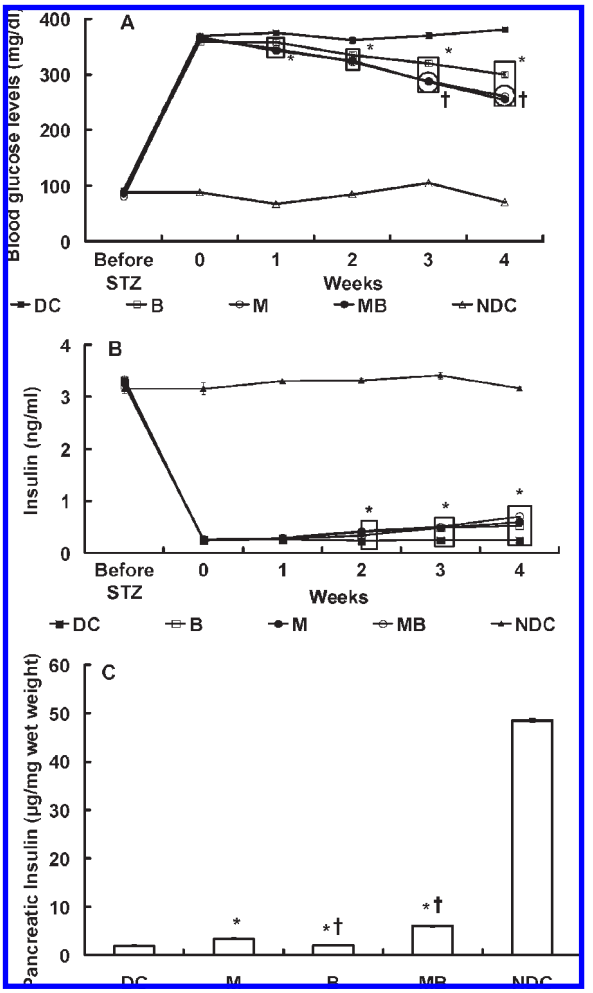

Figure 5. Effects of metformin, baicalin, and metformin + baicalin on (**A**) weekly blood glucose level, (**B**) weekly plasma insulin concentration, and (**C**) pancreatic insulin content in STZ28 diabetic Wistar rats. Values are expressed as mean ± SEM. Each group includes six rats per treatment. *, $p < 0.05$ versus the diabetic control; †, $p < 0.05$ versus the metformin-treated group.

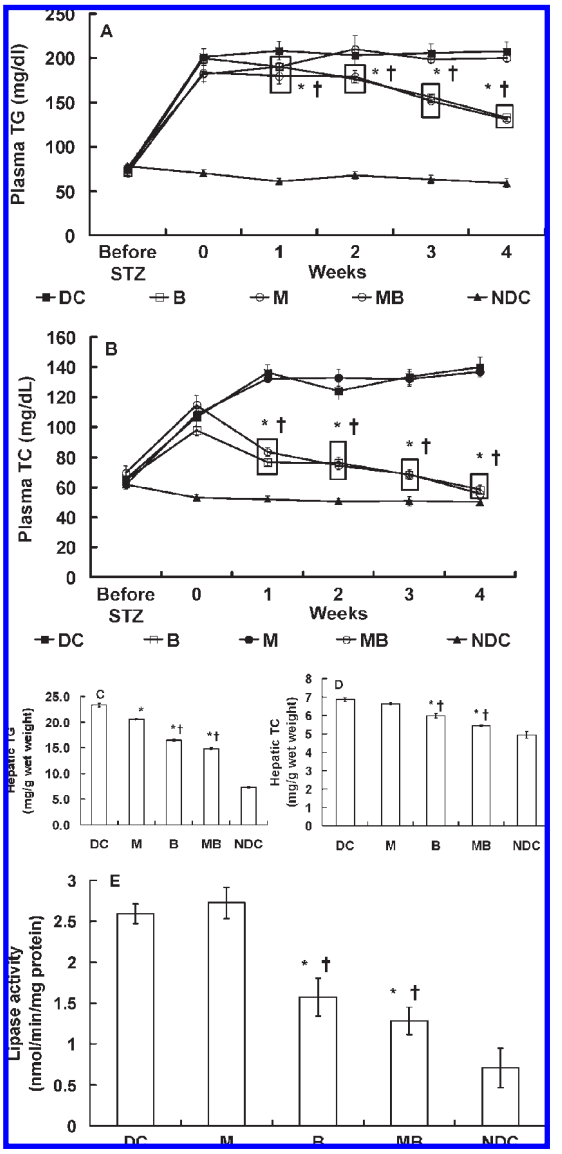

Figure 6. Effects of metformin, baicalin, and metformin + baicalin on (**A**) weekly plasma triglyceride (TG) concentrations, (**B**) weekly plasma total cholesterol (TC) concentrations, (**C**) hepatic TG, (**D**) hepatic TC, and (**E**) hepatic lipase activity in STZ-diabetic Wistar rats. Values are expressed as mean ± SEM. Each group includes six rats per treatment. *, $p < 0.05$ versus the diabetic control; †, $p < 0.05$ versus the metformin-treated group.

indirectly from reduced hyperglycemia, thus ultimately diminishing the oxidative damage (*22*).

Baicalin has an ORAC value of 365 μmol of Trolox equivalents (TE)/g. On a molecular basis, the radical scavenging activity of baicalin is only 0.16 times that of Trolox, the water-soluble vitamin E analogue. In addition, baicalin is a water-soluble compound due to the glucuronic acid group. Therefore, it may be suggested that the decrease of lipid peroxide values may not necessarily be due to the lipid peroxyl radical scavenging capacity but due to an indirect impact on stimulating antioxidant enzyme expressions. It is possible, with reference to the structure of baicalin, the phenolic groups of the A ring may chelate transition metals such as copper and iron to prevent Fenton-like reaction from occurring, thus reducing oxidative stress. Our results illustrate the complexity of antioxidant mechanisms in vivo. In vitro chemistry based assays speak little when it comes to bioactivity of polyphenolic antioxidants (*24*).

The enhancement of antioxidant activity in group MB may have been due to the two separate pathways of reducing oxidative stress of baicalin and metformin. As mentioned before, reduction of plasma glucose levels by metformin would result in a reduction of free radical production through the respective pathways (*4*−*6*). Production of free radicals itself has been known to hinder the secondary and tertiary protein structures of antioxidant enzymes (*6*, *9*). Thus, reduction of free radical production may have resulted in the prevention of damage to the antioxidant enzymes, which was initiated by metformin. In addition, baicalin may have induced the expression of antioxidant enzymes as explained previously. Thus, the combined effect may have resulted in an enhancement of the antioxidant enzyme activities in group MB.

The balance between oxidation and antioxidation (redox status) is known to be an important factor in maintaining a physiologically sound biological system (*23*). An imbalance in the redox status observed in many chronic diseases further confirms the importance of maintaining the physiological redox balance. Although the baicalin and metformin + baicalin treatments had increased the antioxidant activity of the rats in groups B and MB, respectively, and the lipid peroxide concentrations had reduced within the period of treatment, the lipid peroxide concentrations in these groups were still statistically significantly higher compared with the nondiabetic control even after 30 days of

treatment. This observation highlights the extent of oxidative damage induced by the hyperglycemic state and the required direction and level of efficacy in targeting therapeutic applications for diabetes.

Isoprostanes are known to be secondary products of lipid peroxidation; reduction in their levels would indicate a reduction of oxidative stress levels in chronic disease states. The kidney isoprostane contents in groups B and MB were statistically significantly different compared with the diabetic control and group M rats. Plasma CRP is used as an inflammatory marker indicating susceptibility to cardiovascular complications of diabetes. Thus, a reduction in plasma CRP levels would indicate a state of reduced oxidative stress leading to a reduced probability of cardiovascular-related complications. Groups B and MB showed reductions in plasma CRP levels as well, indicating the potential of the combination to reduce such complications in the diabetic state.

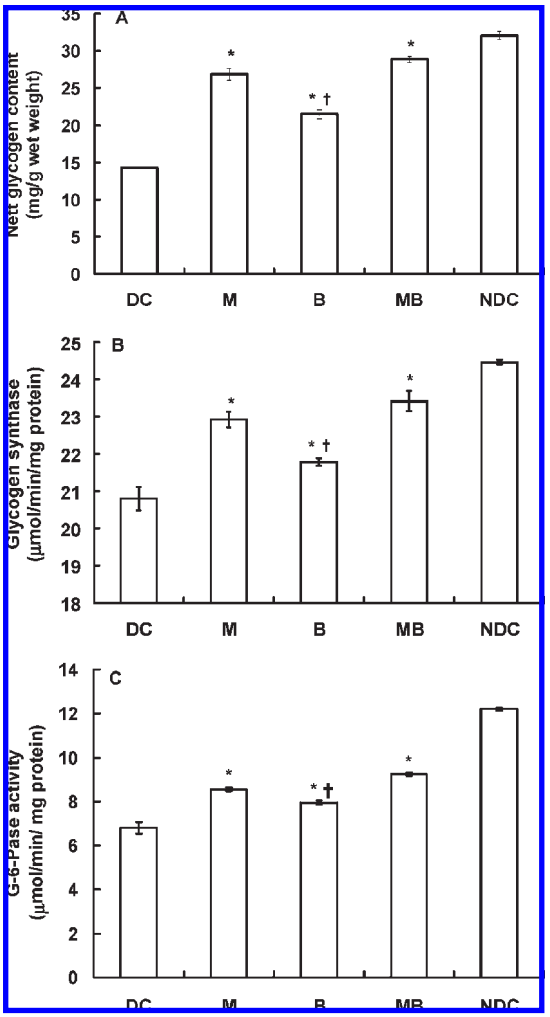

Figure 7. Effects of metformin, baicalin, and metformin + baicalin on (**A**) hepatic glycogen contents, (**B**) hepatic glycogen synthase activity, and (**C**) hepatic glucose-6-phosphatase activity in STZ-diabetic Wistar rats. Values are expressed as mean ± SEM. Each group includes six rats per treatment. ∗, $p < 0.05$ versus the diabetic control; †, $p < 0.05$ versus the metformin-treated group.

Metformin was observed to be more efficient in initiating a hypoglycemic effect than baicalin. Previous studies have shown metformin to stimulate glucose uptake in isolated rat skeletal muscles through the stimulation of AMP-activated protein kinase (AMPK), which is also known to initiate an inhibitory effect on glucose production by hepatocytes (*25*). Reducing the circulating plasma glucose concentrations is one of the most important factors of the equation in the treatment of diabetes. However, reduction of hyperglycemia alone appears to be an insufficient outcome of therapy, as indicated in a number of studies including those on diabetes (*26*, *27*). These studies have shown persisting oxidative stress-induced endothelial dysfunction even when glycemia is normalized. Thus, metformin (and other antihyperglycemic agents) when given alone is insufficient to reverse or prevent such dysfunctions. It appears that direct interventions to reduce oxidative stress are needed in the overall therapy of diabetes. This highlights the importance of flavonoids such as baicalin in the treatment of diabetes.

Baicalin reduced plasma and hepatic TG and TC contents, which was not observed in the metformin-treated group. A recent study has shown polyphenols to stimulate hepatocellular AMPK activity and lipid levels, as well as hyperlipidemia and atherogenesis in type 1 diabetic mice, which is similar in terms of the mechanism to the course of action of metformin. Existing evidence demonstrated that the availability of free fatty acids (FFA) for oxidation by muscles and other tissues may lead to impairment of carbohydrate oxidation as well as to glucose intolerance as seen in obesity and obese diabetics (*27*). In addition, FFA can stimulate hepatic gluconeogenesis and alter pancreatic insulin release as well as pathways of glucose metabolism (*27*). Therefore, the reduction of circulating TG and TC is an important aspect of the treatment of diabetes that was not achieved with metformin in this study. On the other hand, baicalin produced significant reductions in hepatic TG and TC contents. It may be hypothesized that baicalin reduces hyperlipidemia through the activation of hepatocellular AMPK as shown by a recent study in type 1 diabetic mice (*28*).

Administration of STZ is known to initiate pancreatic β-cell death, resulting in an in vivo model for the study of type I diabetes (*29*). However, a few studies have shown the damage by STZ to be partial, leaving some β-cells undamaged (*30*). Thus, the STZ-induced diabetic rat model would tend to show type 2-like features, which also justifies the use of metformin during this study. A significant enhancement in plasma and pancreatic insulin levels was observed in group MB compared with group M. The plasma insulin concentrations of the treatment groups were still significantly lower than the concentrations of the nondiabetic control. The enhanced levels of pancreatic insulin in groups B and MB may be attributed to the protective effects of

Table 1. Effects of Metformin, Baicalin, and Metformin plus Baicalin on Various Biochemical Parameters Measured in Blood Plasma after 30 Days of Treatment ($n = 6$)[a]

biomarker	nondiabetic control	diabetic control	metformin	baicalin	metformin + baicalin
CRP (ng/mL)	20.47 ± 6.79	73.82 ± 1.23	67.14 ± 3.36*	59.54 ± 0.62*,†	56.07 ± 0.43*,†
isoprostane (μg/g)	6.98 ± 1.33	41.87 ± 1.27	37.78 ± 6.97*	34.89 ± 3.86*,†	22.95 ± 5.63*,†
AST (U/L)	21.84 ± 6.88	43.67 ± 13.95	90.87 ± 57.45	23.65 ± 9.78	109.86 ± 36.33
ALT (U/L)	20.77 ± 4.96	41.25 ± 10.30	97.68 ± 59.88	23.97 ± 9.88	102.67 ± 49.37
glucagon (pg/mL)	35.06 ± 0.15	39.82 ± 1.87	34.93 ± 1.20	35.18 ± 0.32	35.20 ± 1.56

[a] Values were statistically significant as compared with (*) the diabetic control ($p < 0.05$) and (†) the metformin-treated group ($p < 0.05$).

increased antioxidant enzyme activities. We are currently investigating the effects of baicalin on genetically modified type 2 diabetic rats, and the results will be disclosed in due course.

In summary, this study has shown baicalin to be an efficient mitigator of hyperglycemia-induced oxidative stress when used alone or in combination with metformin. The importance of maintaining the redox balance as well as reducing elevations in plasma glucose levels has been recognized as an essential and proficient method in combating diabetic macro- and microvascular complications. Thus, the results of this study add strength to the hypothesis that an increase in antioxidant activity plays a role in the prevention of hyperglycemia-induced long-term complications.

ABBREVIATIONS USED

8-OHdG, 8-hydroxydeoxyguanosine; AGE-products, advanced glycation end products; ALT, alanine transaminase; AST, aspartate transaminase; CAT, catalase; cAMP, cyclic adenosine monophosphate; ELISA, enzyme-linked immunosorbent assay; FFA, free fatty acids; G-6-P, glucose-6-phosphate; G-6-Pase, glucose-6-phosphatase; GPx, glutathione peroxidase; HPLC, high-performance liquid chromatography; ORAC, oxygen radical absorbance capacity; PARP, poly-ADP ribose polymerase; PKC, protein kinase-C; ROS, reactive oxygen species; RNS, reactive nitrogen species; SOD, superoxide dismutase; STZ, streptozotocin; TC, total cholesterol; TG, triglycerides.

Supporting Information Available: Details of methodology. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Forbes, J. M.; Coughlan, M. T.; Cooper, M. E. Oxidative stress as a major culprit in kidney disease in diabetes. *Diabetes* **2008**, *57*, 1446–1454.

(2) Rhodes, C. Type II diabetes: a matter of β-cell life or death?. *Science* **2005**, *307*, 380–384.

(3) Hovind, P.; Tarnow, L.; Rossing, K.; Rossing, P.; Eising, S.; Larsen, N.; Binder, C.; Parving, H. H. Decreasing incidence of severe diabetic microangiopathy in type 1 diabetes. *Diabetes Care* **2003**, *26*, 1258–1264.

(4) Rother, K. I. Diabetes treatment—bridging the divide. *N. Engl. J. Med.* **2007**, *356*, 1499–1501.

(5) Brownlee, M. Biochemistry and molecular cell biology of diabetic complications. *Nature (London)* **2001**, *414*, 813–820.

(6) Brownlee, M. The pathobiology of diabetes: a unified mechanism (Banting lecture). *Diabetes* **2005**, *54*, 1615–1625.

(7) Evans, J. L.; Maddux, B. A.; Goldfine, I. D. Antioxidants in diabetic complications and insulin resistance. In *Diabetes: From Research to Diagnosis and Treatment*; Raz, A., Ed.; Martin Dunitz: London, U.K., 2003; pp 479−496.

(8) Coppey, L. J.; Gellett, J. S.; Davidson, E. P.; Dunlap, J. A.; Lund, D. D.; Yorek, M. A. Effect of antioxidant treatment of streptozotocin-induced diabetic rats on endoneurial blood flow, motor nerve conduction velocity, and vascular reactivity of epineurial arterioles of the sciatic nerve. *Diabetes* **2001**, *50*, 1927–1937.

(9) Dierckx, N.; Horvath, G.; van Gils, C.; Vertommen, J.; van de Vliet, J.; De Leeuw, I.; Manuel-y-Keenoy, B. Oxidative stress status in patients with diabetes mellitus: relationship to diet. *Eur. J. Clin. Nutr* **2003**, *57*, 999–1008.

(10) Du, X.; Edelstein, D.; Brownlee, M. Oral benfotiamine plus α-lipoic acid normalises complication-causing pathways in type 1 diabetes. *Diabetologia* **2008**, 1–3.

(11) Skibola, C. F.; Smith, M. T. Potential health impacts of excessive flavonoid intake. *Free Radical Biol. Med.* **2000**, *29*, 375–383.

(12) Ou, B. X.; Huang, D. J.; Hampsch-Woodill, M.; Flanagan, J. When East meets West: the relationship between yin−yang and antioxidation−oxidation. *FASEB J.* **2003**, *17*, 127–129.

(13) Schinella, G. R.; Tournier, H. A.; Prieto, J. M.; Mordujovich , P.; Rios, J. L. Antioxidant activity of anti-inflammatory plant extracts. *Life Sci* **2002**, *70*, 1023–1033.

(14) Ho, J. H.; Kim, D. O.; Soo, J. C.; Dong, H. S.; Chang, Y. L. Potent inhibitory effect of flavonoids from *Scutellaria baicalensis* on amyloid β protein-induced neurotoxicity. *J. Agric. Food Chem.* **2004**, *54*, 4128–4132.

(15) Waisundara, V. Y.; Hsu, A.; Huang, D. J.; Tan, B. K. H. *Scutellaria baicalensis* enhances the anti-diabetic activity of metformin in streptozotocin-induced diabetic Wistar rats. *Am. J. Chinese Med.* **2008**, *36*, 517–540.

(16) Cheng, J. T.; Huang, C. C.; Liu, I. M.; Tzeng, T. F.; Chang, C. J. Novel mechanism for plasma glucose-lowering action for metformin in streptozotocin-induced diabetic rats. *Diabetes* **2006**, *55*, 819–825.

(17) Huang, D. J.; Ou, B. X.; Hampsch-Woodill, M.; Flanagan, J. A.; Prior, R. L. High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *J. Agric. Food Chem.* **2002**, *50*, 4437–4444.

(18) Hay, K. X.; Waisundara, V. Y.; Yong, Z.; Han, M. Y.; Huang, D. J. CdSe nanocrystals as hydroperoxide scavengers: a new approach to highly sensitive quantification of lipid hydroperoxides. *Small* **2007**, *3*, 290–293.

(19) Lee, Y. A.; Kim, Y. J.; Cho, E. J.; Yokozawa, T. Ameliorative effects of proanthocyanidin on oxidative stress and inflammation in streptozotocin-induced diabetic rats. *J. Agric. Food. Chem.* **2007**, *55*, 9395–9400.

(20) Taylor, M. D. Causation of diabetes type 2—the Gordian knot unravels. *N. Engl. J. Med.* **2004**, *350*, 639–641.

(21) Hannon-Fletcher, M. P. A.; O'Kane, M. J.; Moles, K. W.; Weatherup, C.; Barnett, C. R.; Barnett, Y. A. Levels of peripheral blood cell DNA damage in insulin dependent diabetes mellitus human subjects. *Mutat. Res.* **2000**, *460*, 53–60.

(22) Onaran, I.; Guven, G. S.; Ozdas, S. B.; Kanigur, G.; Vehid, S. Metformin does not prevent DNA damage in lymphocytes despite its antioxidant properties against cumene hydroperoxide-induced oxidative stress. *Mutat. Res.* 2006, *611*, 1−8.

(23) Muller, F. L.; Lustgarten, M. S.; Jang, Y.; Richardson, A.; Van Remmen, H. Trends in oxidative aging theories. *Free Radical Biol. Med.* **2007**, *43*, 477–503.

(24) Frankel, E. N.; Finley, J. W. How to standardize the multiplicity of methods to evaluate natural antioxidants. *J. Agric. Food Chem.* **2008**, *56*, 4901–4908.

(25) Zhou, G. C.; Myers, R.; Li, Y.; Chen, Y. L.; Shen, X. L.; Fenyk-Melody, J.; Wu, M.; Ventre, J.; Doebber, T.; Fuji, N.; Musi, N.; Hirshman, M. F.; Goodyear, L. J.; Moller, D. E. Role of AMP-activated protein kinase in mechanism of metformin action. *J. Clin. Invest.* **2001**, *108*, 1167–1174.

(26) The Diabetes Control and Complications Trial Research Group (DCTT). Long-term defects of diabetes and its treatment on cognitive function. *N. Engl. J. Med.* 2007, *356*, 1842−1852.

(27) Unger, R. H.; Orci, L. Diseases of lipid overflow: new perspective on obesity and related disorders. *FASEB J.* **2001**, *15*, 312–321.

(28) Zang, M. W.; Xu, S. Q.; Maitland-Toolan, K. A.; Zuccollo, A.; Hou, X. Y.; Jiang, B. B.; Wierzbicki, M.; Verbeuren, T.; Cohen, R. A. Polyphenols atimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor−deficient mice. *Diabetes* **2006**, *55*, 2180–2191.

(29) Bonnevie-Nielsen, V.; Steffes, M. W.; Lernmark, A. A major loss in islet mass and β-cell function precedes hyperglycemia in mice given multiple doses of streptozotocin. *Diabetes* **1981**, *30*, 424–429.

(30) Garofano, A.; Czernichow, P.; Bréant, B. Impaired β-cell regeneration in perinatally malnourished rats: a study with STZ. *FASEB J.* **2000**, *14*, 2611–2617.

Received for Review September 13, 2008. Revised manuscript received March 2, 2009. Accepted March 26, 2009. This study was supported by start-up grants to D.H. (National University of Singapore Grant R-143-000-244-101) and B.K.-H.T. (Office of Life Sciences, National University of Singapore, Grant R-184-0000117-712).