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Role of maltol in advanced glycation end products and free radicals: in-vitro and in-vivo studies

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Abstract

Inhibitors of advanced glycation end products (AGEs) have potential as preventive agents against diabetic complications. In-vitro AGE inhibitory activity, transition metal chelating, and free radical scavenging activity tests have been used to screen for and identify effective AGE inhibitors. In an ongoing project to elucidate AGE inhibiting active components of heat-processed ginseng, maltol was selected for more detailed investigation. Although there are several lines of evidence concerning the antioxidant activity of maltol, the in-vitro and in-vivo inhibitory effects of maltol on AGE generation have not been evaluated. In the present study, the in-vitro AGE inhibitory effects and free radical scavenging activity of maltol were investigated. In addition, the in-vivo therapeutic potential of maltol against diabetic renal damage was tested using streptozotocin (STZ)-diabetic rats. Maltol showed a stronger AGE inhibitory effect than aminoguanidine, a well known AGE inhibitor. In addition, the hydroxyl radical scavenging activity of maltol on electron spin resonance (ESR) spectrometry was slightly stronger than that of aminoguanidine. Therefore, maltol was found to have stronger in-vitro AGE inhibiting activity compared with aminoguanidine. The administration of 50 mg kg^{-1} per day of maltol suppressed the elevated serum levels of glycosylated protein, renal fluorescent AGEs, carboxymethyllysine, receptors for AGEs, and nuclear factor-kappaB p65 in diabetic control rats. These beneficial effects of maltol against STZ-diabetic renal damage were thought to result from its free radical scavenging and AGE inhibitory effects.

Introduction

Inhibitors of advanced glycation end products (AGEs) have received considerable interest as preventive agents against hyperglycaemia-induced complications. AGEs can alter the structure and function of intracellular and extracellular molecules, increase oxidative stress, and modulate cell activation, signal transduction, and the expression of cytokines and growth factors through receptor-dependent and receptor-independent pathways (Schrijvers et al 2004; Ahmed 2005; Jang et al 2006). Numerous AGE inhibitors have been investigated using in-vitro AGE inhibitory activity tests, but some classes of AGE inhibition are primarily mediated by their transition metal chelating or antioxidant activities (Price et al 2001; Jang et al 2006). Aminoguanidine, a well known AGE inhibitor, can reduce the advanced glycosylation of proteins, and this protective effect is believed to occur via the free radical scavenging activity of aminoguanidine (Courderot-Masuyer et al 1999). Therefore, in-vitro AGE inhibitory activity, transition metal chelating, and free radical scavenging activity tests are thought to be useful screening methods for the design and discovery of therapeutic compounds for diabetic complications.

In our previous studies aimed at identifying the AGE inhibiting active components of heat-processed Panax ginseng, maltol (3-hydroxy-2-methyl-4-pyrone; Figure 1) was one of the major components found to increase during heat processing by Maillard reaction, and showed strong peroxynitrite ($ONOO^-$) and hydroxyl radical (·OH) scavenging activities (Kang et al 2006a, b). In addition, maltol is suggested to be a functional agent that prevents oxidative damage in the brains of mice (Kim et al 2004). Maltol is formed by sucrose pyrolysis or the thermal degradation of starch, and is extensively used in the food, beverage, tobacco, cosmetic and pharmaceutical industries (Johnson et al 1969; Bjeldanes & Chew 1979). The hydroxypyrone structure of maltol is known to show a potent transition metal chelating capability (Yasumoto et al 2004), and maltol with a vanadium structure is known

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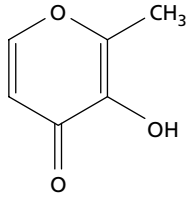

Figure 1 Chemical structure of maltol.

to have an anti-hyperglycaemic effect (Thompson et al 2004). There are several lines of evidence about the antioxidant activity of maltol, and antioxidants are known to protect against glycation-derived free radicals and may have therapeutic potential (Baynes 1991; Ahmed 2005). However, few reports have evaluated the in-vitro and in-vivo inhibitory effects of maltol on AGE generation.

We investigated the in-vitro AGE inhibitory effect and free radical scavenging activity of maltol. In addition, the in-vivo therapeutic potential of maltol against diabetic renal damage was tested in streptozotocin (STZ)-diabetic rats, because in-vivo evidence for a role of AGEs in diabetic kidney disease comes mainly from this animal model (Schrijvers et al 2004).

Materials and Methods

Reagents

Maltol, 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), hydrogen peroxide (H_2O_2), ferrous sulfate ($FeSO_4 \cdot 7H_2O$), and protease inhibitor mixture DMSO solution were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). Aminoguanidine, diethylenetriaminepentaacetic acid (DTPA), phenylmethylsulfonyl fluoride (PMS), STZ, and β-actin were purchased from Sigma Chemical Co. (St Louis, MO, USA). Nuclear factor-kappaB p65 (NF-κB p65), receptors for AGEs (RAGE), and goat anti-rabbit and/or goat anti-mouse IgG horseradish peroxidase conjugated secondary antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The primary polyclonal antibody against carboxymethyllysine (CML) was kindly provided by Dr Nagai of Kumamoto University, Kumamoto, Japan. The other chemicals and reagents used were of high quality and obtained from commercial sources.

In-vitro AGE inhibitory activity

According to the method of Vinson & Howard (1996), 10 mg mL^{-1} bovine serum albumin (BSA) in 50 mM phosphate buffer (pH 7.4) with 0.02% sodium azide (to prevent bacterial growth) was added to glucose (25 mM) and fructose (25 mM) solutions. This reaction mixture was mixed with an aqueous solution of aminoguanidine or maltol (50 and 100 $\mu g\,mL^{-1}$). After incubating at 37°C for 2 weeks, the fluorescent reaction products were assayed on a microplate fluorescence reader, Tecan SPECTROFluor (Tecan UK, Goring-on Thames, UK), with an excitation wavelength of 360 nm and an emission wavelength of 465 nm. The data were expressed in terms of percentage inhibition, calculated from a control measurement of the fluorescence intensity of the reaction mixture with no test sample.

In-vitro •OH scavenging activity

The electron spin resonance (ESR) spectra were recorded on a JES-TE100 ESR spectrometer (JEOL, Tokyo, Japan). The experimental parameters were as follows: temperature, ambient; microwave power, 1.02 mW; modulation frequency, 100 kHz; modulation width, 0.16 mT; sweep width, 5.0 mT; sweep time, 0.5 min; centre field, 339.550 mT; time constant, 0.03 s; receiver gain, 1. DMPO was used as a spin-trapping reagent for ·OH. Mn^{2+} was used as an external standard to calculate the relative amounts from ESR signal intensity. A total of 20 μL of DMPO (1/10 diluted with distilled water, v/v) was mixed with 38 μL of 0.2 mM $FeSO_4 \cdot 7H_2O$ and 37 μL of 1 mM DTPA. The mixture was stirred with 30 μL of an aqueous solution of aminoguanidine or maltol (0.1, 0.5 and 1.0%) and 75 μL of 1 mM H_2O_2. The same amount of thiourea was used as an ·OH scavenging positive control compound. The solutions were transferred to a capillary tube and placed in the ESR spectrometer for measurement. After 5 min, the ESR signal was taken to measure the yield of the inhibition of ·OH by samples. Measurement was repeated twice for each sample. The inhibition of ·OH was determined by the ratio of peak height of the DMPO–OH spin adduct to the signal of Mn^{2+} and compared with the ratio of the control.

Animals and treatment

The animals used in this study were maintained in accordance with Guidelines for Animal Experimentation, approved by the University of Toyama, and were treated humanely. Male Wistar rats (120–130 g) from Japan SLC, Inc. (Hamamatsu, Japan) were used. They were kept in a plastic-bottomed cage under a conventional lighting regimen with a dark night. The room temperature (approx. 25°C) and humidity (approx. 60%) were controlled automatically. They were allowed free access to water and laboratory pellet chow (CLEA Japan Inc., Tokyo, Japan), comprising 24.0% protein, 3.5% lipids and 60.5% carbohydrate. After several days of adaptation, STZ dissolved in citrate buffer (10 mM, pH 4.5) was injected intraperitoneally at a dose of 50 $mg\,kg^{-1}$ following overnight fasting. At 10 days after the injection, the glucose level in blood from the tail vein was determined and the rats were divided into four groups (each group comprised seven rats), avoiding any inter-group differences in blood glucose levels. The control group was given water (vehicle), while the other groups were given maltol orally at a dose of 10, 20 and 50 $mg\,kg^{-1}$ daily using a stomach tube. After administration for 15 consecutive days, urine was collected from the metabolic cage and blood samples were collected from the abdominal aorta. The serum was separated immediately from the blood samples by centrifugation. Subsequently, the renal arteries of each rat were perfused with ice-cold physiological saline (0.9% NaCl, pH 7.4), and the kidneys were removed, quickly frozen and kept at −80°C until analysis.

Assays of serum and urine samples

Serum glucose, total protein, urea nitrogen, and creatinine (Cr) were determined using commercial reagents: Glucose CII-Test Wako and A/G B-Test Wako were obtained from Wako Pure Chemical Industries Ltd, Osaka, Japan; BUN Kainos and CRE-EN Kainos were obtained from Kainos Laboratories Inc., Tokyo, Japan. The serum glycosylated protein and thiobarbituric acid (TBA)-reactive substance levels were measured by reported methods (Fluckiger & Winterhalter 1976; Naito & Yamanaka 1978). Urine component levels were determined as follows: protein by the sulfosalicylic acid method (Sakagishi 1968), and Cr using a commercial reagent (CRE-EN Kainos). Creatinine clearance (C_{Cr}) was calculated on the basis of urinary Cr, serum Cr, urine volume and bodyweight using the following equation: C_{Cr} (mL min^{-1} (kg body weight)$^{-1}$) = (urinary Cr (mg dL^{-1}) × urine volume (mL)/serum Cr (mg dL^{-1})) × (1000/bodyweight (g)) × (1/1440 (min)).

Determination of renal levels of AGEs

The renal levels of AGEs were determined by the method of Nakayama et al (1993). Minced kidney tissue was delipidated with chloroform and MeOH (2:1, v/v) overnight. After washing, the tissue was homogenized with 0.1 M NaOH, followed by centrifugation at 8000 *g* for 15 min at 4°C. The amounts of AGEs in these alkali-soluble samples were determined by measuring the fluorescence at an emission wavelength of 465 nm and excitation wavelength of 360 nm using a Tecan SPECTRAFluor. Data were expressed in arbitrary units: units (mg of protein)$^{-1}$. One unit was defined as the sample fluorescence compared with that of BSA in 0.1 M NaOH. Protein levels were evaluated by the micro-biuret method (Itzhaki & Gill 1964) with BSA as the standard.

Electrophoretic pattern analysis of urinary protein

Urinary protein samples from rats (8 μg) were loaded onto a 10% acrylamide gel and subjected to sodium dodecyl sulfate polyamide gel electrophoresis (SDS-PAGE), and the protein bands were stained for 30 min with Coomassie brilliant blue R-250 in a distilled water/MeOH/acetic acid (4.5:5:0.5, v/v/v) mixture and destained overnight in 7% acetic acid. The stained polyamide gels were scanned using a densitometer (AE-6911CX; ATTO Corp., Tokyo, Japan).

Western blotting

Renal cortical sections were homogenized with ice-cold lysis buffer (pH 7.5) containing 137 mM NaCl, 20 mM Tris-HCl, 1% Tween-20, 10% glycerol, 1 mM PMS, and the protease inhibitor mixture DMSO. Samples were then centrifuged at 2000 *g* for 10 min at 4°C. The protein concentration of tissue was determined using a Bio-Rad protein assay kit and BSA as a standard, and then immunoblotting was carried out. For the determination of NF-κB p65, CML and RAGE protein levels in the kidney, 30 μg of protein from each sample was electrophoresed through 8% or 12% SDS-PAGE. Separated proteins were electrophoretically transferred to a nitrocellulose membrane, blocked with 5% skim milk solution for 3 h at 4°C, and then incubated with primary antibodies overnight at 4°C. After the blots were washed, they were incubated with goat anti-rabbit and/or goat anti-mouse IgG horseradish peroxidase conjugated secondary antibody for 90 min at room temperature. Each antigen–antibody complex was visualized using ECL Western Blotting Detection Reagents (Amersham, New Jersey, USA) and detected by chemiluminescence with LAS-1000 plus (FUJIFILM, Japan). Band densities were determined by Scion image software (Scion Corporation, Frederick, MD, USA) and quantified as the ratio to β-actin.

Statistical analysis

The results for each group are expressed as mean ± s.e. values. The effect on each parameter was examined using one-way analysis of variance. Individual differences between groups were evaluated using Dunnett's test and those at $P < 0.05$ were considered significant.

Results

In-vitro AGE inhibition and free radical scavenging activity

Figure 2A shows the effects of aminoguanidine and maltol on the production of AGEs. Aminoguanidine inhibited protein glycation to approximately 71% and 51% at concentrations of 50 and 100 μg mL^{-1}, respectively. In the case of maltol, it more strongly inhibited protein glycation, to approximately 31% and 23% at concentrations of 50 and 100 μg mL^{-1}, respectively, compared with aminoguanidine. Figure 2B shows the in-vitro ·OH scavenging activity of maltol with positive control compounds. Aminoguanidine (1.0%) inhibited ·OH production to approximately 35%; it was more strongly inhibited, to approximately 32%, by the addition of maltol. However, none of these effects were stronger than those of thiourea, the ·OH scavenging positive control compound.

Changes in physico-metabolic symptoms

Table 1 shows the effects of maltol on changes in physico-metabolic symptoms associated with diabetes over the 15-day experimental period. The body weight gain of STZ-induced diabetic rats was significantly lower than that of normal rats. However, there were no significant changes in body weight between diabetic control and maltol-treated groups. On the other hand, the kidney weight of diabetic control rats was significantly higher than that of normal rats, but it was significantly reduced by the administration of maltol at a dose of 50 mg kg^{-1}. In addition, the levels of food intake, water intake, and urine volumes were markedly elevated in diabetic control rats. Food intake showed no changes between diabetic control and maltol-treated groups. However, water intake and urine excretion levels were significantly reduced by daily administration of maltol at 50 mg kg^{-1}.

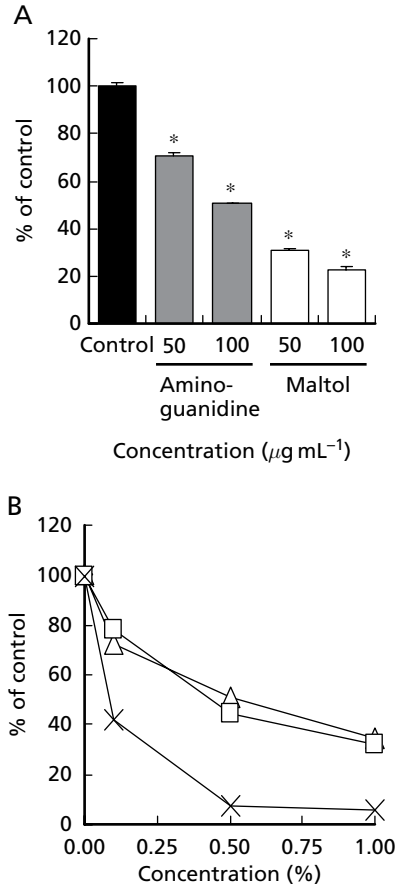

Figure 2 In-vitro advanced glycation end product inhibitory activity (A) and in-vitro ·OH-scavenging activity (B) of maltol with positive control compounds. □, Maltol; △, aminoguanidine; ×, thiourea. *$P < 0.01$, significantly different compared with the control value.

Biochemical features of serum and urine

Table 2 shows the effects of maltol on general biochemical parameters of serum and urine. Diabetic control rats showed a remarkably higher blood glucose concentration than normal rats, while the elevated glucose level was significantly reduced in diabetic rats given 50 mg kg^{-1} per day of maltol. The serum glycosylated protein and TBA-reactive substance levels of the diabetic control rats were also significantly increased compared with normal rats, but were significantly decreased by the administration of maltol at a dose of 50 mg kg^{-1}. In the case of the serum urea nitrogen level, it was increased from 20.5 mg dL^{-1} in normal rats to 31.6 mg dL^{-1} in diabetic control rats, but there were no significant changes with maltol administration. On the other hand, there were no significant changes in serum Cr, urinary protein, and C_{Cr} levels among normal, diabetic control and maltol-treated groups.

Renal levels of AGEs

The effects of maltol on renal levels of AGEs is shown in Table 3. The renal level of AGEs in diabetic control rats was approximately 1.9-times higher than in normal rats, but it was lowered, dose dependently, by maltol administration, and significantly reduced by 50 mg kg^{-1} per day of maltol administration.

Electrophoretic patterns of proteinuria

Figure 3 shows the effects of maltol on the proteinuria pattern in STZ-induced diabetic rats. The albumin band is strongly expressed at approximately 65 kDa in Figure 3A, and its band intensities are shown graphically in Figure 3B. The albumin band intensity of diabetic control rats was about 1.6-times higher than that of normal rats, but it was decreased significantly by the administration of 50 mg kg^{-1} maltol.

Western blotting

The protein expression related to AGE formation in renal tissue is shown in Figure 4. There were significant increases in NF-κB p65, CML and RAGE expression in diabetic control rats compared with normal rats. The elevated NF-κB p65 level (65 kDa) of the diabetic control group was significantly decreased by the administration of 50 mg kg^{-1} per day of maltol (Figure 4A). In addition, CML accumulation (~50- and 37-kDa un-identified proteins) and RAGE expression (46 kDa) in diabetic control rats were ~2.0-, 1.6-, and 2.0-times higher than those of normal rats, respectively. However, the elevated CML levels at ~50 and 37 kDa were

Table 1 Physico-metabolic symptoms

Group	Dose (mg kg^{-1} per day)	Body weight			Kidney weight (g/100 g body weight)	Food intake (g per day)	Water intake (mL per day)	Urine volume (mL per day)
		Initial (g)	Final (g)	Gain (g)				
Normal	–	241.4 ± 4.0**	311.2 ± 6.1**	69.8 ± 3.6**	0.70 ± 0.02**	21.0 ± 0.2**	44.3 ± 1.8**	13.7 ± 0.8**
Diabetic								
Control	–	204.4 ± 4.5	232.6 ± 7.7	28.3 ± 4.4	1.09 ± 0.03	30.8 ± 0.3	139.5 ± 3.9	101.4 ± 4.1
Maltol	10	205.3 ± 5.5	233.8 ± 8.4	28.5 ± 3.9	1.03 ± 0.04	29.8 ± 0.6	131.0 ± 3.6	94.4 ± 3.6
Maltol	20	204.5 ± 5.3	234.0 ± 5.1	29.0 ± 2.9	1.01 ± 0.06	30.0 ± 0.4	122.0 ± 5.5*	88.0 ± 5.1*
Maltol	50	205.9 ± 3.2	234.1 ± 5.8	28.3 ± 3.6	0.99 ± 0.03*	30.3 ± 0.4	118.3 ± 4.9*	84.6 ± 5.1*

Data are expressed as mean ± s.e. *$P < 0.05$, **$P < 0.01$ compared with diabetic control rats.

Table 2 Biochemical features of serum and urine

Item	Normal	Diabetic rats			
		Control	Maltol (10 mg)	Maltol (20 mg)	Maltol (50 mg)
Serum glucose (mg dL^{-1})	145 ± 3**	531 ± 12	519 ± 17	514 ± 19	494 ± 9*
Serum glycosylated protein (nmol (mg protein)$^{-1}$)	14.4 ± 0.2**	23.9 ± 2.1	22.0 ± 0.6	19.5 ± 1.3	19.3 ± 0.4*
Serum TBA-reactive substance (nmol mL^{-1})	1.76 ± 0.13*	2.83 ± 0.31	2.36 ± 0.22	2.16 ± 0.26	1.87 ± 0.18*
Serum urea nitrogen (mg dL^{-1})	20.5 ± 0.3**	31.6 ± 1.1	31.7 ± 0.7	31.1 ± 1.3	29.8 ± 0.9
Serum creatinine (mg dL^{-1})	0.33 ± 0.02	0.36 ± 0.02	0.35 ± 0.02	0.35 ± 0.01	0.36 ± 0.02
Urinary protein (mg per day)	9.3 ± 0.6	10.9 ± 0.9	10.2 ± 0.9	9.8 ± 0.7	9.8 ± 0.7
C_{Cr} (mL min^{-1}/kg body weight)	6.86 ± 0.41	6.02 ± 0.37	6.28 ± 0.32	6.44 ± 0.43	6.40 ± 0.49

TBA, thiobarbituric acid; C_{Cr}, creatinine clearance. Data are expressed as mean ± s.e. *$P < 0.05$, **$P < 0.01$ compared with diabetic control rats.

Table 3 Renal levels of advanced glycation end products (AGEs)

Group	Dose (mg kg^{-1} per day)	AGEs (AU)
Normal	–	0.36 ± 0.02**
Diabetic		
Control	–	0.70 ± 0.10
Maltol	10	0.65 ± 0.13
Maltol	20	0.58 ± 0.12
Maltol	50	0.53 ± 0.08*

Data are expressed as mean ± s.e. *$P < 0.05$, **$P < 0.01$ compared with diabetic control rats.

significantly decreased in the group administered with 50 mg kg^{-1} per day of maltol (Figure 4B). In addition, the elevated RAGE level was also significantly reduced by the administration of maltol at 50 mg kg^{-1} (Figure 4C).

Discussion

When glucose and other reactive carbonyl compounds react non-enzymatically with proteins, lipids or nucleic acids, Schiff bases and Amadori products are formed. Additional rearrangement and modification leads to the generation of diverse AGEs. These AGEs can alter the structure and function of intracellular and extracellular molecules, increase oxidative stress, and modulate cell activation, signal transduction and the expression of cytokines and growth factors through receptor-dependent and receptor-independent pathways (Wendt et al 2003; Williams 2003; Schrijvers et al 2004). The inhibition of AGEs has received considerable interest because it is closely related to the prevention of diabetic complications (Courderot-Masuyer et al 1999; Price et al 2001). In our ongoing project to elucidate AGE-inhibiting active components of heat-processed ginseng, maltol was selected for more detailed investigation. Heat-processed ginseng showed greater free radical scavenging activity than conventional white or red ginseng, and a protective effect against STZ-induced diabetic renal damage in rats by inhibiting AGEs and oxidative stress. The content of maltol was significantly

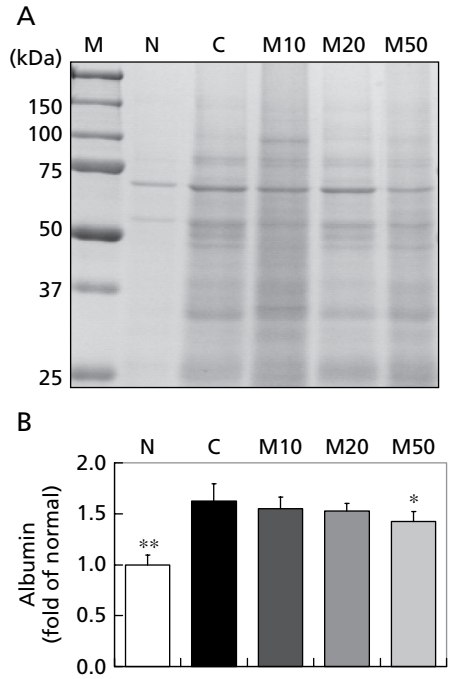

Figure 3 SDS-PAGE pattern of urinary protein. M, protein marker; N, normal rats; C, diabetic control rats; M10, diabetic rats treated with 10 mg kg^{-1} maltol per day; M20, diabetic rats treated with 20 mg kg^{-1} maltol per day; M50, diabetic rats treated with 50 mg kg^{-1} maltol per day; *$P < 0.05$, **$P < 0.01$, significantly different compared with diabetic control rats.

increased by the heat processing of ginseng and showed strong $ONOO^-$ and ·OH scavenging activity (Kang et al 2006a, b).

Maltol is known to be formed by sucrose pyrolysis or the thermal degradation of starch, and is also a typical marker of the Maillard reaction (Johnson et al 1969; Davidek et al 2003). In addition, maltol is widely used as a flavouring agent in food and beverages (Bjeldanes & Chew 1979), and shows a potent metal-chelating action owing to its hydroxyketone structure (Figure 1) (Yasumoto et al 2004). Maltol was used to increase the ease of vanadate absorbance because vanadate

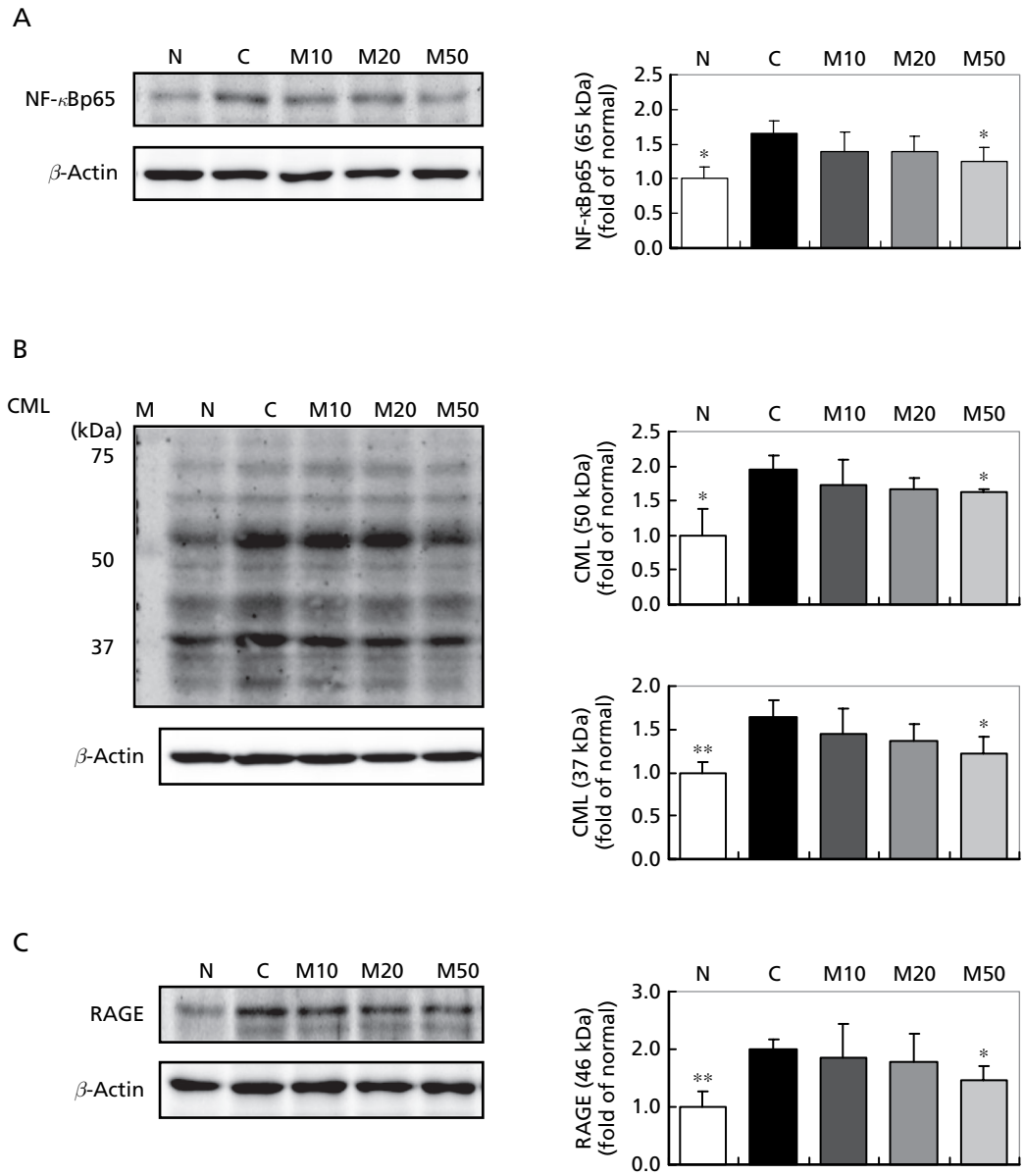

Figure 4 Effects of maltol on nuclear factor-kappaB p65 (NF-κB p65), carboxymethyllysine (CML) and receptors for advanced glycation end product (RAGE) expression. N, normal rats; C, diabetic control rats; M10, diabetic rats treated with 10 mg kg^{-1} maltol per day; M20, diabetic rats treated with 20 mg kg^{-1} maltol per day; M50, diabetic rats treated with 50 mg kg^{-1} maltol per day; *$P < 0.05$, **$P < 0.01$, significantly different compared with diabetic control rats.

is known to have insulin-mimicking effects but it is poorly absorbed from the gastrointestinal tract into the blood, and this vanadium complex with maltol is known to have insulin-enhancing or glucose-lowering properties with a lower dose (Yuen et al 1997; Saatchi et al 2005). In addition, maltol is known to exhibit a neuroprotective effect against oxidative stress (Kim et al 2004). Therefore, maltol was thought to have potential inhibitory effects against AGE generation in-vitro and in-vivo because antioxidants are known to protect against glycation-derived free radicals and may have therapeutic potential (Baynes 1991; Ahmed 2005). However, few studies have evaluated the inhibitory effect of maltol on AGE generation, and so this was investigated in the present study.

As shown by our in-vitro data, maltol exhibited a stronger inhibitory effect against glucose-induced AGE generation than aminoguanidine, a well-known AGE inhibitor. In addition, the ·OH scavenging activity of maltol was slightly stronger than that of aminoguanidine, and this effect was interpreted to be important because ·OH scavenging activity in ESR is mediated by the transition metal chelating and free radical scavenging activity of the compounds (Huang et al 2005). Therefore, maltol was evaluated to have a stronger in-vitro AGE inhibitory activity than aminoguanidine, and the effect of maltol on STZ-induced diabetic renal damage in rats was investigated because in-vivo evidence for a role of AGEs in diabetic kidney disease comes mainly from studies in

STZ-diabetic rats (Schrijvers et al 2004). In particular, spin-trapping agents such as phenyl *N*-tert-butylnitrone are known to react with and stabilize free radical species, significantly reducing the severity of hyperglycaemia in STZ-induced diabetic rats (Tabatabaie et al 1997; Evans et al 2002).

From the physico-metabolic symptoms, biochemical features of serum and urine, and SDS-PAGE pattern of urinary protein, early diabetic renal changes occurred, but not advanced nephropathy, in the diabetic rats used in this study. There was a significant decrease in body weight gain, increases in kidney weight, water intake, urine volume, serum glucose and urea nitrogen, and urinary albumin levels in diabetic control rats (Tables 1 and 2; Figure 3), but signs of advanced diabetic renal changes, such as urinary protein and C_{Cr}, were not significantly changed in diabetic rats compared with normal rats. In addition, the diabetic rats used in this study showed significant increases in serum glycosylated protein and TBA-reactive substance levels. However, there were significant decreases in kidney weight, water intake, urine volume, serum glucose, glycosylated protein, TBA-reactive substance, and urinary albumin levels in the group administered 50 mg kg^{-1} per day of maltol compared with diabetic control rats. Therefore, maltol administration was thought to have beneficial effects in this early stage of diabetic renal damage, and this effect has important implications in preventing the progression from early to advanced stages. Although the identification and management of diabetic kidney disease in the early stage is important, the majority of people have no symptoms until the disease is very advanced (Levin 2001).

In the case of renal levels of AGE, a significant accumulation of fluorescent AGE levels in the kidney of diabetic rats compared with normal rats was observed. In people with diabetes and/or chronic renal failure, AGEs that accumulate in the kidney are responsible for pathological changes, including increased kidney weight, glomerular hypertrophy, glomerular basement membrane thickening and progressive albuminuria (Vlassara et al 1994). Moreover, AGEs stimulate free radical mechanisms and induce membrane peroxidation, which in turn increase membrane permeability. Therefore, AGE accumulation in the kidney has been regarded as an index of progressive renal damage in diabetic nephropathy. Maltol (50 mg kg^{-1} per day) significantly decreased the renal fluorescent AGE level (Table 3), suggesting that it would inhibit oxidative damage and irreversible renal damage caused by the protein glycation reaction in diabetes. The effects of maltol on the other non-fluorescent AGEs were not determined in the present study and so remain to be clarified.

Regarding hyperglycaemia-induced renal function parameters and tissue damage, Western blot analyses of protein expression related to AGE formation were performed. NF-κB is normally present in the cytoplasm of eukaryotic cells as an inactive complex with the inhibitory protein, IκB. When cells are exposed to various external stimuli, such as reactive oxygen species or AGEs, IκB undergoes rapid phosphorylation with subsequent ubiquitination, leading to the proteosome-mediated degradation of this inhibitor. Activation of NF-κB has been suggested to participate in diabetes and its complications (Surh et al 2001; Ahmed 2005). Although the transcriptional activation of NF-κB using a protein–DNA binding assay was not measured in the present study, the NF-κB p65 protein level was overexpressed in the diabetic rat kidney, and the overexpression of NF-κB p65 was significantly inhibited by the administration of 50 mg kg^{-1} per day of maltol (Figure 4A). On the other hand, CML, a major AGE in human tissues, is known to be a marker of cumulative oxidative stress and is involved in the development of diabetic nephropathy (Horie et al 1997). In addition, the activation of RAGE by CML results in the activation of NF-κB and the production of pro-inflammatory cytokines (Yan et al 1994; Ahmed 2005). There were significantly increased CML and RAGE levels in diabetic control rats compared with normal rats, but they were significantly reduced by the administration of 50 mg kg^{-1} per day of maltol (Figure 4B and C). These findings imply that the beneficial effect of maltol was mediated by the inhibition of AGE generation and the deactivation of NF-κB.

Although a comparison of the effects of maltol with aminoguanidine in STZ-diabetic rats was not conducted in this study, the AGE inhibitory effect of maltol is thought to be similar to that of aminoguanidine. Aminoguanidine treatment is known not to show a strong antihyperglycaemic effect, but it attenuates the rise in albuminuria, renal CML and RAGE levels (Soulis-Liparota et al 1991; Huang et al 1998; Sugimoto et al 1999), as shown in the present study of maltol. Moreover, aminoguanidine is also reported to have significant antioxidant activity by increasing superoxide dismutase and catalase activity, but it also has side-effects such as flu-like symptoms, gastrointestinal disturbances, and anaemia (Kedziora-Kornatowska & Luciak 1998; Ahmed 2005). Studies on the effect of maltol at a higher dose or more advanced stage of diabetic renal damage compared with aminoguanidine are needed, but the potential inhibitory effect of maltol against AGE generation in-vitro and in-vivo has been determined in this study.

In summary, maltol was found to be a stronger in-vitro AGE inhibitor than aminoguanidine. In STZ-diabetic rats, the administration of 50 mg kg^{-1} per day of maltol suppressed serum glycosylated protein, renal fluorescent AGE, CML, RAGE and NF-κB p65 levels. The beneficial effects of maltol on STZ-diabetic renal damage were thought to have resulted from its free radical scavenging and AGE inhibitory effects.

References

Ahmed, N. (2005) Advanced glycation endproducts - role in pathology of diabetic complications. *Diabetes Res. Clin. Pract.* **67**: 3–21

Baynes, J. W. (1991) Role of oxidative stress in development of complications in diabetes. *Diabetes* **40**: 405–412

Bjeldanes, L. F., Chew, H. (1979) Mutagenicity of 1,2-dicarbonyl compounds: maltol, kojic acid, diacetyl and related substances. *Mutat. Res.* **67**: 367–371

Courderot-Masuyer, C., Dalloz, F., Maupoil, V., Rochette, L. (1999) Antioxidant properties of aminoguanidine. *Fundam. Clin. Pharmacol.* **13**: 535–540

Davidek, T., Clety, N., Devaud, S., Robert, F., Blank, I. (2003) Simultaneous quantitative analysis of Maillard reaction precursors and products by high-performance anion exchange chromatography. *J. Agric. Food Chem.* **51**: 7259–7265

Evans, J. L., Goldfine, I. D., Maddux, B. A., Grodsky, G. M. (2002) Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr. Rev.* **23**: 599–622

Fluckiger, R., Winterhalter, K. H. (1976) In vitro synthesis of hemoglobin AIc. *FEBS Lett*. **71**: 356–360

Horie, K., Miyata, T., Maeda, K., Miyata, S., Sugiyama, S., Sakai, H., van Ypersole de Strihou, C., Monnier, V. M., Witztum, J. L., Kurokawa, K. (1997) Immunohistochemical colocalization of glycoxidation products and lipid peroxidation products in diabetic renal glomerular lesions. Implication for glycoxidative stress in the pathogenesis of diabetic nephropathy. *J. Clin. Invest*. **100**: 2995–3004

Huang, D., Ou, B., Prior, R. L. (2005) The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem*. **53**: 1841–1856

Huang, Y., Lin, S., Zhou, J. (1998) Gene expression of receptor for advanced glycosylation end products and its modulation by aminoguanidine in diabetic kidney tissue. *Chin. Med. J*. **111**: 698–704

Itzhaki, R. F., Gill, D. M. (1964) A micro-biuret method for estimating proteins. *Anal. Biochem*. **9**: 401–410

Jang, D. S., Kim, J. M., Lee, Y. M., Kim, Y. S., Kim, J. H., Kim, J. S. (2006) Puerariafuran, a new inhibitor of advanced glycation end products (AGEs) isolated from the roots of *Pueraria lobata*. *Chem. Pharm. Bull*. **54**: 1315–1317

Johnson, R. R., Alford, E. D., Kinzer, G. W. (1969) Formation of sucrose pyrolysis products. *J. Agric. Food Chem*. **17**: 22–24

Kang, K. S., Kim, H. Y., Pyo, J. S., Yokozawa, T. (2006a) Increase in the free radical scavenging activity of ginseng by heat-processing. *Biol. Pharm. Bull*. **29**: 750–754

Kang, K. S., Kim, H. Y., Yamabe, N., Nagai, R., Yokozawa, T. (2006b) Protective effect of sun ginseng against diabetic renal damage. *Biol. Pharm. Bull*. **29**: 1678–1684

Kedziora-Kornatowska, K., Luciak, M. (1998) Effect of aminoguanidine on lipid peroxidation and activities of antioxidant enzymes in the diabetic kidney. *Biochem. Mol. Biol. Int*. **46**: 577–583

Kim, Y. B., Oh, S. H., Sok, D. E., Kim, M. R. (2004) Neuroprotective effect of maltol against oxidative stress in brain of mice challenged with kainic acid. *Nutr. Neurosci*. **7**: 33–39

Levin, A. (2001) Identification of patients and risk factors in chronic kidney disease-evaluating risk factors and therapeutic strategies. *Nephrol. Dial. Transplant*. **16**: 57–60

Naito, C., Yamanaka, T. (1978) Lipid peroxides in atherosclerotic diseases. *Jpn. J. Geriat*. **15**: 187–191

Nakayama, H., Mitsuhashi, T., Kuwajima, S., Aoki, S., Kuroda, Y., Itoh, T., Nakagawa, S. (1993) Immunochemical detection of advanced glycation end products in lens crystallins from streptozotocin-induced diabetic rat. *Diabetes* **42**: 345–350

Price, D. L., Rhett, P. M., Thorpe, S. R., Baynes, J. W. (2001) Chelating activity of advanced glycation end-product inhibitors. *J. Biol. Chem*. **276**: 48967–48972

Saatchi, K., Thompson, K. H., Patrick, B. O., Pink, M., Yuen, V. G., McNeill, J. H., Orvig, C. (2005) Coordination chemistry and insulin-enhancing behavior of vanadium complexes with maltol $C_6H_6O_3$ structural isomers. *Inorg. Chem*. **44**: 2689–2697

Sakagishi, Y. (1968) Total protein. In: Saito, M., Kitamura, M., Niwa, M. (eds) *Rinsho Kagaku Bunseki II*. Tokyo Kagaku Dojin, Tokyo, pp 115–142

Schrijvers, B. F., Vriese, A. S. D., Flyvbjerg, A. (2004) From hyperglycemia to diabetic kidney disease: the role of metabolic, hemodynamic, intracellular factors and growth factors/cytokines. *Endocr. Rev*. **25**: 971–1010

Soulis-Liparota, T., Cooper, M., Papazoglou, D., Clarke, B., Jerums, G. (1991) Retardation by aminoguanidine of development of albuminuria, mesangial expansion, and tissue fluorescence in streptozocin-induced diabetic rat. *Diabetes* **40**: 1328–1334

Sugimoto, H., Shikata, K., Wada, J., Horiuchi, S., Makino, H. (1999) Advanced glycation end products-cytokine-nitric oxide sequence pathway in the development of diabetic nephropathy: aminoguanidine ameliorates the overexpression of tumor necrosis factor-α and inducible nitric oxide synthase in diabetic rat glomeruli. *Diabetologia* **42**: 878–886

Surh, Y. J., Chun, K. S., Cha, H. H., Han, S. S., Keum, Y. S., Park, K. K., Lee, S. S. (2001) Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kappa B activation. *Mutat. Res*. **480–481**: 243–268

Tabatabaie, T., Kotake, Y., Wallis, G., Jacob, J. M., Floyd, R. A. (1997) Spin trapping agent phenyl *N*-tert-butylnitrone protects against the onset of drug-induced insulin-dependent diabetes mellitus. *FEBS Lett*. **407**: 148–152

Thompson, K. H., Chiles, J., Yuen, V. G., Tse, J., McNeill, J. H., Orvig, C. (2004) Comparison of anti-hyperglycemic effect amongst vanadium, molybdenum and other metal maltol complexes. *J. Inorg. Biochem*. **98**: 683–690

Vinson, J. A., Howard, T. B. (1996) Inhibition of protein glycation and advanced glycation end products by ascorbic acid and other vitamins and nutrients. *J. Nutr. Biochem*. **7**: 659–663

Vlassara, H., Striker, L. J., Teichberg, S., Fuh, H., Li, Y. M., Steffes, M. (1994) Advanced glycation end products induce glomerular sclerosis and albuminuria in normal rats. *Proc. Natl Acad. Sci. USA* **91**: 11704–11708

Wendt, T., Tanji, N., Guo, J., Hudson, B. I., Bierhaus, A., Ramasamy, R., Arnold, B., Nawroth, P. P., Yan, S. F., D'Agati, V., Schmidt, A. M. (2003) Glucose, glycation, and RAGE: implications for amplification of cellular dysfunction in diabetic nephropathy. *J. Am. Soc. Nephrol*. **14**: 1383–1395

Williams, M. E. (2003) New therapies for advanced glycation end product nephrotoxicity: current challenges. *Am. J. Kidney Dis*. **41**: S42–S47

Yan, S. D., Schmidt, A. M., Anderson, G. M., Zhang, J., Brett, J., Zou, Y. S., Pinsky, D., Stern, D. (1994) Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. *J. Biol. Chem*. **269**: 9889–9897

Yasumoto, E., Nakano, K., Nakayachi, T., Morshed, S. R., Hashimoto, K., Kikuchi, H., Nishikawa, H., Kawase, M., Sakagami, H. (2004) Cytotoxic activity of deferiprone, maltol and related hydroxyketones against human tumor cell lines. *Anticancer Res*. **24**: 755–762

Yuen, V. G., Caravan, P., Gelmini, L., Glover, N., McNeill, J. H., Setyawati, I. A., Zhou, Y., Orvig, C. (1997) Glucose-lowering properties of vanadium compounds: comparison of coordination complexes with maltol or kojic acid as ligands. *J. Inorg. Biochem*. **68**: 109–116