ORIGINAL ARTICLE

Relationship between peroxisome proliferator-activated receptor- γ activation and the ameliorative effects of ascochlorin derivatives on type II diabetes

Junji Magae^{1,2}, Mie Tsuruga², Ayako Maruyama^{2,3}, Chiharu Furukawa², Shuji Kojima³, Hirohiko Shimizu⁴ and Kunio Ando⁴

Peroxisome proliferator-activated receptor- γ (PPAR- γ) is a crucial factor in the development of insulin resistance associated with type II diabetes. We previously found that 4-*O*-carboxymethyl ascochlorin, a derivative of ascochlorin, ameliorates diabetes and activates PPAR- γ . Here, we compared the relationship between the amelioration of type II diabetes in *db/db* mice lacking leptin receptor, and PPAR- γ activation by 4-*O*-carboxymethyl-ascochlorin, as well as by 4-*O*-methyl-ascochlorin, a derivative that does not activate PPAR- γ . Administration of these compounds significantly reduces blood glucose in a dose-dependent manner, whereas blood cholesterol is significantly elevated in 4-*O*-carboxymethyl-ascochlorin-treated mice but is significantly decreased in 4-*O*-methyl-ascochlorin-treated mice. Pioglitazone, a potent PPAR- γ agonist with a thiazolidinedione structure, reduces glucose but elevates cholesterol blood levels. These results suggest that ascochlorin derivatives ameliorate diabetes through a mechanism that is probably independent of PPAR- γ activation, although PPAR- γ activation could be partially involved in the ameliorative effect in certain derivatives.

The Journal of Antibiotics (2009) 62, 365–369; doi:10.1038/ja.2009.43; published online 26 June 2009

Keywords: ascochlorin; AS-6; diabetes; MAC; PPAR- γ

INTRODUCTION

There are two types of diabetes mellitus. Type I, or insulin-dependent diabetes mellitus, is characterized by a progressive loss of insulin-producing β -cells in the pancreas by an autoimmune mechanism, and the resulting insulin deficiency produces severe hyperglycemia.¹ Destruction of β -cells in type I diabetes is known to be caused by apoptosis induced through an oxidative reaction initiated by nitric oxide generated in β -cells.^{2–4} Type II diabetes is developed through insulin resistance and the subsequent insufficiency of β -cell compensation.⁵ Although the mechanism causing insulin resistance is not clearly understood, the involvement of a nuclear hormone receptor, peroxisome proliferator-activated receptor- γ (PPAR- γ), is suggested. PPAR- γ has a crucial function in the differentiation of adipocytes, and agonists of PPAR- γ induce a differentiation of preadipocyte cell lines and ameliorate type II diabetes by improving insulin resistance.⁶ PPAR- γ is activated by thiazolidinediones, originally synthesized as a drug to treat hyperlipidemia and insulin resistance. These compounds bind to PPAR- γ with high affinity and enhance the differentiation of preadipocyte cells into mature adipocytes.

Ascochlorin is a prenylphenol antibiotic, originally isolated as an antiviral agent produced by an incomplete fungus, *Ascochyta visiae*.^{7,8}

Ascochlorin and its derivatives exhibit a large variety of physiological activities, including hypolipidemic activity,^{9,10} suppression of hypertension,¹¹ amelioration of type I and II diabetes,^{12,13} immunomodulation^{14,15} and antitumor activity through the phagocyte-mediated activation of innate immunity.^{15,16} In addition, ascochlorin and ascofuranone suppress tumor invasion *in vitro* and selectively suppress AP-1 activity in human renal carcinoma cells, as well as its downstream targets, such as matrix metalloproteinase-9, through suppression of the ERK1/2 signaling pathway.^{17,18} 4-*O*-Methyl-ascochlorin (MAC), a methylated derivative of ascochlorin, induces apoptosis in a variety of leukemia cell lines through a mechanism that is distinct from conventional apoptosis.¹⁹ Ascochlorin selectively kills estrogen receptor (ER)-negative breast cancer cells, partly through the induction of apoptosis.²⁰ A proteome analysis of ascochlorin-treated human osteosarcoma cells shows a decrease in the expression of several genes in the mitogen-activated protein kinase (MAPK)-signaling cascade, including epidermal growth factor receptor and ERK-1/2.²¹ Ascochlorin and ascofuranone moderate oxidative phosphorylation by inhibiting ubiquinone-dependent electron transport in isolated mitochondria,^{22–24} and it is suggested that the antiviral activity of ascochlorin and the macrophage activation of

¹Radiation Safety Research Center, Central Research Institute of Electric Power Industry, 2-11-1 Iwado Kita, Komae-shi, Tokyo, Japan; ²Department of Biotechnology, Institute of Research and Innovation, 1201 Takada, Kashiwa, Chiba, Japan; ³Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki Noda-shi, Chiba, Japan and ⁴NRL Pharma Inc., KSP, 3-2-1 Sakado, Takatsu-ku, Kawasaki, Japan

Correspondence: Dr J Magae, Nuclear Technology Research Laboratory, Radiation Safety Research Center, Central Research Institute of Electric Power Industry, 2-11-1 Iwado Kita, Komae-shi, Tokyo 201-8511, Japan.

E-mail: jmagae@sannet.ne.jp

Received 19 December 2008; revised 18 May 2009; accepted 19 May 2009; published online 26 June 2009

ascofuranone result from this inhibitory activity on mitochondrial respiration.^{22,23,25}

These compounds also modulate the activity of nuclear hormone receptors. Ascochlorin activates human ER, suggesting that mechanisms other than those involving the respiratory chain contribute to their physiological activities. Some derivatives, including 4-*O*-carboxymethyl-ascochlorin (AS-6), activate PPAR- γ and induce a differentiation of preadipocytes.^{26,27} AS-6 inhibits tumor necrosis factor- α -stimulated nuclear factor- κ B activity and the inflammatory molecular expression of rat vascular smooth muscle cells in a manner dependent on PPAR- γ activation.²⁸ In this paper, we compare the relationship between PPAR- γ activation and the ameliorative effect on type II diabetic *db/db* mice lacking the leptin receptor. Our results suggest that the amelioration of type II diabetes by AS-6 is consistent with a mechanism of PPAR- γ activation, whereas MAC may act by a mechanism that is independent of PPAR- γ .

MATERIALS AND METHODS

Mice

Eight-week-old male *db/db* mice and age-matched normal male littermates (*db/m*) were purchased from Sankyo Lab Service Inc. (Tokyo, Japan). The mice were given a diet of commercial food pellets and tap water *ad libitum*. Animal studies were conducted in the animal facility of the Institute of Research and Innovation under approved protocols in accordance with institutional guidelines for laboratory animal care.

Chemicals

AS-6 and MAC were obtained from Chugai Pharmaceutical Co. (Tokyo, Japan). Pioglitazone was obtained from Takeda Pharmaceutical Co. (Tokyo, Japan). The compounds were dissolved in dimethylsulfoxide at 10 mg ml⁻¹ as stock solutions and appropriately diluted with medium for addition to the culture medium. For *in vivo* administration, mice were fed a pellet diet containing one of the drugs *ad libitum*.

Cell culture

U2OS, a human osteosarcoma, was provided by C-L Wu (MGH Cancer Center, Charlestown, MA, USA). Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum at 37 °C in a humidified incubator under 10% CO₂ atmosphere.

Plasmids

Expression vectors of the transactivation domain of PPAR- γ fused with a Gal4-DNA-binding domain,²⁹ and the luciferase reporter plasmid containing a

Gal4-binding site upstream of SV-40 basic promoter³⁰ were kind gifts from S Kato (Institute of Molecular and Cellular Bioscience, University of Tokyo, Tokyo, Japan) and S Inamoto (Institute of Research and Innovation, Kashiwa, Japan). pCMV- β -galactosidase was provided by NH Heintz (Department of Pathology, University of Vermont, Burlington, VT, USA).

Proliferation assay

U2OS cells (1 $\times$ 10⁴ cells) were cultured with drugs for 24 h in microplate wells, and [³H]thymidine (0.5 μ Ci/well) was added 4 h before harvesting.²⁷ Incorporated radioactivity was determined by a liquid scintillation counter.

Reporter assay

U2OS cells (1 $\times$ 10⁶ cells in a 10 cm dish) were transfected with pCMV- β -Gal (4 μ g), an expression vector of the Gal4-fused PPAR- γ (2 μ g), and a corresponding reporter plasmid (4 μ g) using a liposome-based transfection kit (Fugene, Roche Diagnostics, Tokyo, Japan) as described previously.^{26,27} Cells were cultured for 24 h. Luciferase activity (Roche Diagnostics) and β -galactosidase activity (Promega, Madison, WI, USA) in the cell lysates were determined with commercial assay kits.

Biochemical analysis of plasma

Plasma samples were obtained from blood samples containing 10 U ml⁻¹ heparin sulfate. Total cholesterol (Kyowa Medex, Tokyo Japan) and glucose (GL-5, Kainos, Tokyo, Japan) in the samples were measured with a commercial assay kit. Insulin (Morinaga, Yokohama, Japan) and leptin (R&D Systems, Minneapolis, MN, USA) were measured by an enzyme-linked immunosorbent assay with a commercial assay kit according to the manufacturer's instructions.

Statistical analysis

The results of animal experiments were statistically analyzed with a two-tailed *t*-test, judging the significance by a *P*-value less than 0.05.

RESULTS

Activation of PPAR- γ by AS-6 but not by MAC

4-*O*-Methyl-ascochlorin (MAC) and 4-*O*-carboxymethyl-ascochlorin (AS-6) are prototypes of the ascochlorin derivatives that we have synthesized (Figure 1a). MAC has hypolipidemic activity,^{9,10} whereas AS-6 ameliorates diabetes *in vivo*.^{12,13} MAC inhibits cell growth, induces apoptosis and activates no nuclear receptors except PXR, whereas AS-6 is less toxic and selectively activates PPAR- γ .²⁷ The agonistic activity of these two compounds on PPAR- γ was compared in U2OS cells transfected with the expression plasmid for PPAR- γ fused with the Gal4-DNA-binding domain, together with the luciferase reporter plasmid containing a Gal4-binding site in the promoter

Figure 1 Differential activation of PPAR- γ by AS-6 and MAC. (a) The structure of AS-6 and MAC. (b) U2OS cells transfected with expression plasmids for Gal4-fused PPAR- γ β -galactosidase and a luciferase-reporter plasmid for Gal4 were cultured in the presence of the drug for 24 h. Luciferase activity normalized with β -galactosidase activity was determined. Each point represents the average stimulation index compared with non-stimulated controls in independent triplicate experiments. (c) U2OS cells were cultured in the presence of the drug for 24 h. Cell proliferation was determined by [³H] thymidine uptake over 4 h at the termination of culture. Each point represents an average percent uptake compared with non-stimulated controls in independent triplicate experiments. AS-6, open circles; MAC, filled circles; pioglitazone, open squares.

region. AS-6 at 30 μ M increased luciferase activity 30-fold 24 h after transfection, whereas MAC had no agonistic activity in this reporter system (Figure 1b). One of the thiazolidinedione compounds, pioglitazone, activates PPAR- γ at concentrations above 1 μ M, and produced more than a 100-fold activation at 30 μ M. In contrast, the three compounds comparably suppressed cell proliferation as measured by [3 H] thymidine incorporation 24 h after addition of the drug (Figure 1c). The drugs had only a negligible effect on cell proliferation at a concentration below 10 μ M, and completely suppressed cell [3 H] thymidine incorporation at 100 μ M.

Amelioration of type II diabetes in *db/db* mice

To evaluate the ameliorative effects of AS-6 and MAC, 8-week-old *db/db* mice were fed a 0.2–0.4% drug-containing diet for five consecutive days, and blood glucose levels were determined after overnight fasting. In *db/db* mice, blood glucose levels were elevated to a level three times that of age-matched normal *db/m* mice (Figure 2a). Administration of AS-6 and MAC significantly reduced elevated glucose levels. This effect was more pronounced in mice fed a MAC-containing diet. Mice fed a diet containing 0.8% AS-6, 0.4% MAC or 0.8% MAC showed glucose levels lower than normal. Administration of pioglitazone also

reduced blood glucose levels to normal. Polydipsia is one of the characteristics of diabetes patients, and *db/db* mice consumed much more drinking water than did normal mice (Figure 2b). The administration of AS-6 and MAC, as well as that of pioglitazone, reduced polydipsia. *db/db* mice also consumed more food than did normal mice, and food consumption was decreased in mice fed a drug-containing diet (Figure 2c). Mice consumed 50% less in the 0.8%-AS-6 group and in the 0.4 and 0.8%-MAC groups, compared with control *db/db* mice.

db/db mice are obese as well as diabetic, and their body weight (36.9 ± 1.0) is much heavier than that of age-matched normal mice (21.8 ± 0.2), although weight gain during the 5-day experiment was not significantly different between normal and *db/db* mice (Figure 2d). Weight gain during the experiment period was significantly reduced in 0.4 and 0.8% AS-6-fed mice and in all the MAC-treated mice. In contrast, weight gain was significantly increased in mice fed the 0.2% AS-6-containing diet or the 0.1% pioglitazone-containing diet, suggesting that activation of PPAR- γ increases body weight, whereas blood glucose levels are reduced to normal. A lower dose of MAC (0.1%) did not increase weight gain and had no significant effect on blood glucose (data not shown). The administration of a diet containing more than 1% MAC or AS-6 killed the animals. Blood levels of

Figure 2 *In vivo* ameliorative effects of AS-6 and MAC on type II diabetes in *db/db* mice. *Db/db* mice (three mice/group in each cage) were starved overnight, and then fed an AS-6 or MAC-containing diet for 5 days. After overnight fasting, plasma was isolated from heparin-treated blood samples, and concentrations of glucose (a), insulin (e), total cholesterol (f) and leptin (g) were determined. Water (b) and food consumption (c) in each group were recorded during the experiment. Body weights were compared before and after the experiment after overnight fasting (d). Each value for panels a, d–g represents the mean $\pm$ s.d. *Statistically significant compared with control group ($P < 0.05$).

insulin and leptin are dramatically increased in *db/db* mice compared with those in normal mice (Figures 2e and f), which is characteristic of type II diabetes in *db/db* mice genetically lacking leptin receptors.²⁹ AS-6 and MAC, as well as pioglitazone, reduced leptin and insulin levels in blood, although, because of the variation among mice, these differences were not statistically significant.

Modulation of blood cholesterol in *db/db* mice

Ascochlorin derivatives affect lipid metabolism in mice. To confirm the hypocholesterolemic effects of AS-6 and MAC, blood cholesterol levels in *db/db* mice were measured. We found significantly higher cholesterol concentrations in *db/db* mice (123.8 ± 27.1 mg per 100 ml) compared with that in normal mice (58.4 ± 6.6 mg per 100 ml) (Figure 2g). Administration of AS-6 significantly increased the cholesterol levels up to more than 200 mg per 100 ml. In contrast, the administration of MAC significantly reduced cholesterol levels to below 100 mg per 100 ml, the level comparable with that in normal mice. Pioglitazone, similar to AS-6, significantly increased blood cholesterol levels, suggesting that the activation of PPAR- γ increases blood cholesterol in type II diabetic model mice.

DISCUSSION

Since we isolated ascochlorin from fungal broth in 1968, we have studied the modulation of carbohydrate and lipid metabolism by ascochlorin and its derivatives.^{9–13} AS-6 is one of the derivatives in which we found an amelioration of diabetes.^{12,13} Treatment with AS-6 dramatically suppresses polydipsia, polyuria and glucosuria in *db/db* mice,¹³ type II diabetes mice lacking leptin receptors.³¹ The pancreatic islets isolated from AS-6-treated *db/db* mice release much more insulin in response to glucose loading. The combined treatment with insulin and AS-6 synergistically decreases serum glucose. Moreover, AS-6, similar to thiazolidinediones, which ameliorate type II diabetes through the improvement of insulin resistance,⁶ activates PPAR- γ and induces a differentiation of preadipocyte cell lines.²⁶ These results suggest that AS-6 restores sensitivity and responsiveness to insulin in *db/db* mice through the activation of PPAR- γ . Agonistic activity on PPAR- γ is not associated with all the derivatives we studied.²⁷ MAC is a derivative with much weaker agonistic activity than AS-6. Thus, it is of interest to compare the *in vivo* efficacy of AS-6 and MAC on type II diabetes. In this study, we found that MAC and AS-6 have comparable ameliorative activity on type II diabetes in *db/db* mice. They similarly reduced hyperglycemia, hyperinsulinemia and polydipsia, comparable with pioglitazone, in spite of the negligible PPAR- γ agonistic activity of MAC *in vitro*. Treatment with pioglitazone significantly increases body weight and plasma cholesterol. Treatment with a lower dose of AS-6 (diet containing 0.2% AS-6) also increased body weight and plasma cholesterol. In contrast, MAC reduces weight gain and plasma cholesterol. These results suggest that MAC ameliorates type II diabetes in a manner independent of PPAR- γ , whereas AS-6 does so through mechanisms dependent on and independent of PPAR- γ . Consistent with these results, AS-6 is also effective in treating streptozotocin-induced type I diabetes, caused by insulin deficiency resulting from drug-mediated β -cell destruction.¹²

Several possibilities could be proposed for the mechanism of PPAR- γ -independent amelioration of type II diabetes mediated by ascochlorin derivatives. The most distinguishing characteristic of MAC among ascochlorin derivatives *in vitro* is apoptosis induction.¹⁹ It is reported that many regulators of apoptosis have an important function in energy metabolism. For instance, a proapoptotic Bcl-2 family member, Bad, associates with glucokinase in mitochondria, and a dysfunction of the protein impairs glucose clearance in glucose

tolerance tests.³² Survival kinases Akt and Pim also regulate cellular metabolism, in part, through a phosphatidylinositol-3-kinase/mammalian target of rapamycin-dependent mechanism.³³ Although type II diabetes is characterized by insulin resistance,⁵ the ability of pancreatic islets to secrete insulin is gradually decreased as the disease progresses, because the islets degenerate as a result of the toxic effects of reactive oxygen species.^{34,35} Thus, antioxidants reduce blood glucose levels, improve glucose clearance and increase insulin secretion in response to glucose loading.³⁵ A mitochondrial NADH reductase, apoptosis-inducing factor, released from mitochondria during the apoptotic process leading to caspase-independent apoptosis, serves as a free radical scavenger to prevent apoptosis in cerebellar granule cells.³⁶ It is also possible that ascochlorin derivatives directly participate in a cellular oxidative reaction because they react with the electron transport chain in mitochondria.^{23–25}

Finally, we could not exclude the trivial possibility that a suppression of food intake might reduce hyperglycemia and glucosuria, because higher doses of MAC and AS-6 significantly reduced body weight, whereas a low dose of AS-6 and pioglitazone rather increased weight gain. Although the administration of AS-6 and MAC by mixing in the diet could not determine the exact doses, we could not detect significant efficacy by direct administration methods. Oral administration of AS-6 or MAC up to 1 g kg⁻¹ using a gastric feeding needle failed to demonstrate a significant ameliorative effect on diabetes as well as toxicity, presumably because of their bioavailability. The intraperitoneal administration of AS-6 and MAC every other day showed no significant efficacy on diabetes at 25 mg kg⁻¹, and caused toxic death at 50 mg/kg. An improvement of toxicity and bioavailability through chemical structure modification or through the drug delivery method is absolutely necessary to develop AS-6 and MAC as clinical drugs for the treatment of diabetes patients.

ACKNOWLEDGEMENTS

This study was in part supported by grants from the New Energy and Industrial Technology Development Organization (NEDO).

- 1 Yoon, J. W., Jun, H. S. & Santamaria, P. Cellular and molecular mechanisms for the initiation and progression of β cell destruction resulting from the collaboration between macrophages and T cells. *Autoimmunity* **27**, 109–122 (1998).
- 2 O'Brien, A., Harmon, B. V., Cameron, D. P. & Allan, D. J. Apoptosis is the mode of β -cell death responsible for the development of IDDM in the nonobese diabetic mouse. *Diabetes* **46**, 750–757 (1997).
- 3 Kurrer, M. O., Pakala, S. V., Hanson, H. L. & Katz, D. J. β -cell apoptosis in T cell-mediated autoimmune diabetes. *Proc. Natl Acad. Sci. USA* **94**, 213–218 (1997).
- 4 Corbett, J. A., Swetland, M. A., Wang, J. L., Lancaster, Jr. J. R. & McDaniel, M. L. Nitric oxide mediates cytokine-induced inhibition of insulin secretion by human islets of Langerhans. *Proc. Natl Acad. Sci. USA* **90**, 1713–1735 (1993).
- 5 DeFronzo, R. A. Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes genes. *Diabetes Rev.* **5**, 177–269 (1997).
- 6 Rosen, E. D., Walkey, C. J., Puigserver, P. & Spiegelman, B. M. Transcriptional regulation of adipogenesis. *Genes Dev.* **14**, 1293–1307 (2000).
- 7 Tamura, G., Suzuki, S., Takatsuki, A., Ando, K. & Arima, K. Ascochlorin, a new antibiotic, found by the paper-disc agar-diffusion method. I. Isolation, biological and chemical properties of ascochlorin. (Studies on antiviral and antitumor antibiotics. I). *J. Antibiot.* **21**, 539–544 (1968).
- 8 Nawata, Y., Ando, K., Tamura, G., Arima, K. & Iitake, Y. The molecular structure of ascochlorin. *J. Antibiot.* **2**, 511–512 (1969).
- 9 Hosokawa, T., Sawada, M., Ando, K. & Tamura, G. Enhanced excretion of fecal neutralsterols and the hypercholesterolemic properties of 4-O-methylascochlorin. *Agric. Biol. Chem.* **44**, 2461–2468 (1980).
- 10 Hosokawa, T., Sawada, M., Ando, K. & Tamura, G. Alteration of cholesterol metabolism by 4-O-methylascochlorin in rats. *Lipids* **16**, 433–438 (1981).
- 11 Hosokawa, T., Okutomi, T., Sawada, M., Ando, K. & Tamura, G. Unusual concentration of urine and prevention of polydipsia by fungal prenylphenols in DOCA hypertensive rats. *Eur. J. Pharmacol.* **69**, 429–438 (1981).

- 12 Hosokawa, T., Ando, K. & Tamura, G. An ascochlorin derivative, AS-6 potentiates insulin action in streptozotocin diabetic mice and rats. *Agric. Biol. Chem.* **46**, 775–781 (1982).
- 13 Hosokawa, T., Ando, K. & Tamura, G. An ascochlorin derivative, AS-6, reduces insulin resistance in the genetically obese diabetic mice. *Diabetes* **34**, 267–274 (1982).
- 14 Magae, J. *et al.* *In vitro* effects of an antitumor antibiotic, ascofuranone, on the murine immune system. *Cancer Res.* **46**, 1073–1078 (1986).
- 15 Magae, J. *et al.* Antitumor and antimetastatic activity of an antibiotic, ascofuranone and activation of phagocytes. *J. Antibiot.* **40**, 959–965 (1988).
- 16 Magae, J., Hosokawa, T., Ando, K., Nagai, K. & Tamura, G. Antitumor protective property of an isoprenoid antibiotic, ascofuranone. *J. Antibiot.* **35**, 1547–1552 (1982).
- 17 Hong, S. H. *et al.* Ascochlorin inhibits matrixmetalloproteinase-9 expression by suppressing AP-1 mediated-gene expression through the ERK1/2 signaling pathway: inhibitory effects of ascochlorin on the invasion of renal carcinoma cells. *J. Biol. Chem.* **280**, 25202–25209 (2005).
- 18 Cho, H. J. *et al.* Ascofuranone suppresses PMA-mediated matrix metalloproteinase-9 gene activation through the Ras/Raf/MEK/ERK- and Ap1-dependent mechanisms. *Carcinogenesis* **28**, 1104–1110 (2007).
- 19 Tsuruga, M. Characterization of 4-*O*-methyl-ascochlorin-induced apoptosis in comparison with typical apoptotic inducers in human leukemia cell lines. *Apoptosis* **9**, 429–435 (2004).
- 20 Sakaguchi, K. *et al.* Selective cytotoxicity of ascochlorin in ER-negative human breast cancer cell lines. *Biochem. Biophys. Res. Commun.* **329**, 46–50 (2005).
- 21 Kang, J. H. *et al.* Proteome analysis of responses to ascochlorin in a human osteocarcinoma cell line by 2-D gel electrophoresis and MALDI-TOF MS. *J. Proteome Res.* **5**, 2620–2631 (2006).
- 22 Takatsuki, A., Tamura, G. & Arima, K. Antiviral and antitumor antibiotics. XIV. Effects of ascochlorin and other respiration inhibitors on multiplication of Newcastle disease virus in cultured cells. *Appl. Microbiol.* **17**, 825–829 (1969).
- 23 Magae, J. *et al.* Effects of microbial products on glucose consumption and morphology of macrophages. *Biosci. Biotechnol. Biosci.* **57**, 1628–1631 (1993).
- 24 Minagawa, N., Meguro, K., Sakajo, S. & Yoshimoto, A. Effects of ascofuranone on the mitochondria isolated from *Hansenulaanomala*. *Biosci. Biotechnol. Biochem.* **58**, 1334–1335 (1994).
- 25 Ashikaga, T. *et al.* Selective induction of interleukin-1 production and tumor killing activity of macrophages through apoptosis by the inhibition of oxidative respiration. *Biosci. Biotechnol. Biochem.* **62**, 1115–1121 (1998).
- 26 Togashi, M. *et al.* PPAR γ activation and adipocyte differentiation induced by AS-6, a prenylphenol antidiabetic antibiotic. *J. Antibiot.* **55**, 417–422 (2002).
- 27 Togashi, M. *et al.* Ascochlorin derivatives as ligands for nuclear hormone receptors. *J. Med. Chem.* **46**, 4113–4123 (2003).
- 28 Kang, J. H. *et al.* Ascochlorin suppresses oxLDL-induced MMP-9 expression by inhibiting the MEK/ERK signaling pathway in human THP-1 macrophages. *J. Cell Biochem.* **102**, 506–514 (2007).
- 29 Yanase, T. *et al.* Differential expression of PPAR γ_1 and γ_2 isoforms in human adipose tissue. *Biochem. Biophys. Res. Commun.* **233**, 320–324 (1997).
- 30 Harding, H. P. & Lazar, M. A. The monomer-binding orphan receptor Rev-Erb represses transcription as a dimer on a novel direct repeat. *Mol. Cell Biol.* **15**, 4791–4802 (1995).
- 31 Chen, H. *et al.* Evidence that the diabetes gene encodes the leptin receptor: Identification of a mutation in the leptin receptor gene in *db/db* mice. *Cell* **84**, 491–495 (1996).
- 32 Danial, N. N. *et al.* BAD and glucokinase reside in a mitochondrial complex that integrates glycolysis and apoptosis. *Nature* **424**, 952–956 (2003).
- 33 Amaravadi, R. & Thompson, C. B. The survival kinases Akt and Pim as potential pharmacological targets. *J. Clin. Invest.* **115**, 2618–2624 (2005).
- 34 Kaneto, H. *et al.* Reducing sugars trigger oxidative modification and apoptosis in pancreatic β -cells by provoking oxidative stress through the glycation reaction. *Biochem. J.* **320**, 855–863 (1996).
- 35 Kaneto, H. *et al.* Beneficial effects of antioxidants in diabetes. Possible protection of pancreatic β -cells against glucose toxicity. *Diabetes* **48**, 2398–2406 (1999).
- 36 Klein, J. A. *et al.* The harlequin mouse mutation downregulates apoptosis-inducing factor. *Nature* **419**, 367–374 (2002).