매실의 인플루엔자와 헬리코박터 파이로리의 억제효과 및 항당뇨 효과

Japanese Apricot: a Natural Source for Anti-*Helicobacter pylori*, Anti-hyperglycemic and Anti-influenza Virus Agents

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Ⅰ. Introduction

It has been reported that Japanese apricot (*Prunus mume* Sieb. et Zucc) has several biological activities that have human health benefits, such as improving blood fluidity (1), inhibition of growth signals of vascular smooth muscle cells induced by angiotensin II, possibly leading to reduction of cardiovascular diseases (2), suppression of *Helicobacter pylori*-induced glandular stomach lesions in Mongolian gerbils (3), and an anti-hyperglycemic effect on animal model (4).

Prunus mume with common names including Japanese apricot is an Asian tree species classified in the *Armeniaca* section of the genus *Prunus*. The fruits of Japanese apricot are taken in foods as umeboshi, Bainiku-ekisu, pickled Japanese apricot, ume liquor and ume-based soft drinks. The fruit has been known to have various biological activities, and the fruit has been prescribed medicine for disorders of the stomach and intestines, quick recovery from fatigue, cough and diarrhea in Chinese traditional prescriptions (5,6). However, there have been few reports providing proof that components of Japanese apricot are effective against diseases. Three important diseases confronting the world have the possibility of being controlled by Japanese apricot: (i) cancer of the stomach (gastric cancer), a leading cancer-related killer that has been shown to have a strong correlation with *Helicobacter pylori (H. pylori)* infection, (ii) diabetes, one of the most common chronic diseases that is correlated with hyperglycemia, and (iii) influenza, the most widely occurring respiratory disease that is commonly associated with influenza A viruses, which sometimes cause a pandemic generally leading to severe illness with a high mortality rate.

In this review, we shed light on (i) inhibition of

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H. pylori motility by (+)-syringaresinol from unripe Japanese apricot (7), (ii) anti-hyperglycemic effects of plum in a model of obesity and type 2 diabetes, Wistar fatty rat (4), and (iii) multiple inhibitory effects on pandemic influenza A (H1N1) virus by mumefural and its derivatives isolated from Japanese apricot fruit juice concentrate (8) in order to drive searching for more active compounds and development of lead structures in the Japanese apricot for control of the above diseases globally threatening human health.

II. Inhibition of *H. pylori* motility by (+)-syringaresinol from unripe Japanese apricot

H.pylori was isolated from the gastric antrum of chronic gastritis patients. *H. pylori* is closely associated with gastritis and peptic ulcers and is even a bacterial risk factor for gastric cancer (9-12). The bacteria can grow in an acidic environment due to its ability to produce urease, an enzyme breaking down urea to carbon dioxide and ammonia (NH_3), a weak base, which neutralizes gastric acid by reacting with proton (H^+). Therefore, eradication of the bacteria and inhibition of the production of urease are important for treatment of patients with gastroduodenal diseases.

H. pylori has a spiral shape with multiple flagella providing strong motility, and the motility is required for colonization of the stomach that causes gastric inflammation (gastritis) (13,14). Thus, one possible approach for prevention of colonization of *H. pylori* would be to inhibit *H. pylori* motility.

Miyazawa et al. (7) reported the isolation and identification of an inhibitor of *H. pylori* motility from unripe Japanese apricot. The authors isolated (+)-syringaresinol (Fig.1), which is purified from methanol extract of unripe Japanese apricot. This compound showed a potent inhibitory effect in an *in vitro H. pylori* motility assay; it inhibited >90% of *H. pylori* motility at a concentration of 500 ㎍/ml, and the IC_{50} value was 50 ㎍/ml (Fig. 2). Tsutsui et al. reported inhibitory effects of rabeprazple and its thioether derivative on *H. pylori* motility due to their actions as anti-acid agents (proton pump inhibitors) (15). However, the mechanism by which *H. pylori* motility is inhibited by (+)-syringaresinol is unknown, and more studies are needed to clarify the mechanism.

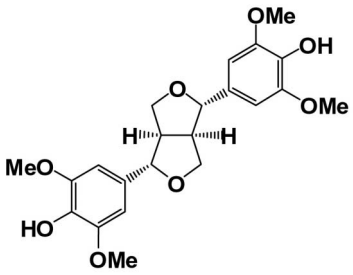

Figure 1. Chemical structure of (+)-syringaresinol (7). Original figure was courteously provided by Dr. Miyazawa.

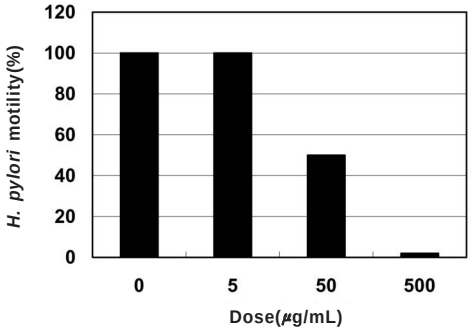

Figure 2. Inhibitory effect of (+)-syringaresinol on *H. pylori* motility (7). Original figure was courteously provided by Dr. Miyazawa.

Ⅲ. Anti-hyperglycemic effects of plum in a model of obesity and type 2 diabetes, Wistar fatty rat

Type 2 diabetes, a leading cause of death in the developed world, is clinically evidenced as hyperglycemia, strongly associated with metabolic disturbances by obesity. The metabolic disturbances underlie induction of insulin resistance, a state of reduced responsiveness to normal circulating levels of insulin regulating carbohydrate and fat metabolisms in the body.

Utsunomiya and Yamakawa et al. (4) evaluated the effects of plum ekisu (concentrated plum juice) on blood glucose and investigated its mechanism in obese diabetic Wistar fatty rats and diabetes (db/db) mice. Oral administration of plum ekisu to Wistar fatty rats decreased blood glucose and plasma triglyceride concentrations in comparison with those in water-treated controls. Plum treatment for 2 weeks reduced areas under the curve (AUCs) for glucose and insulin during a glucose tolerance test (Fig. 3). These findings were confirmed in db/db mice; plum decreased the AUCs and blood glucose during an insulin tolerance test.

Recently, adiponectin has been attracting attention due to its role in regulation of insulin sensitivity and development of insulin resistance (16). Plum treatment appeared to significantly increase plasma adiponectin concentrations (Fig. 4) and peroxisome proliferator-activated receptor (PPAR)-γ mRNA expression in adipose tissue from Wistar fatty rats, suggesting that plum increases circulating adiponectin via induction of PPARγ. The increase of circulating adiponectin is believed to increase insulin sensitivity by stimulating insulin secretion and/or regulating energy homeostasis (16). The antidiabetic effect of concentrated plum juice in these rats may thus be mediated in part by adiponectin.

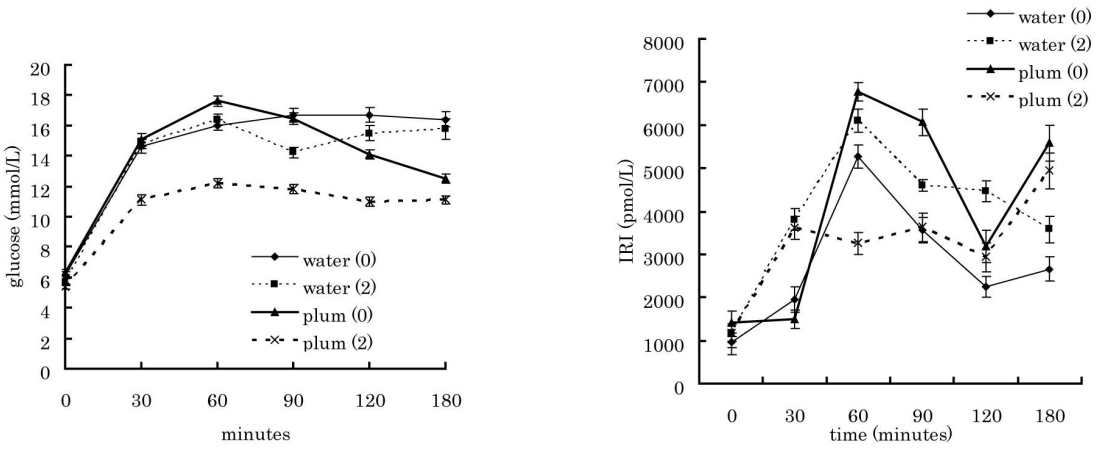

Figure 3. Effect of concentrated plum juice on oral glucose tolerance in Wistar fatty rats (original figure courteously provided by Dr. Yamakawa). Glucose concentration (left panel) and insulin concentration (right panel) after an oral glucose challenge. Values are means ± SE for eight animals per group. 0, before treatment; 2, after treatment for 2 weeks. *P<0.05, **P<0.001 vs. water-treated Wistar fatty rats. IRI: immunoreactive insulin.

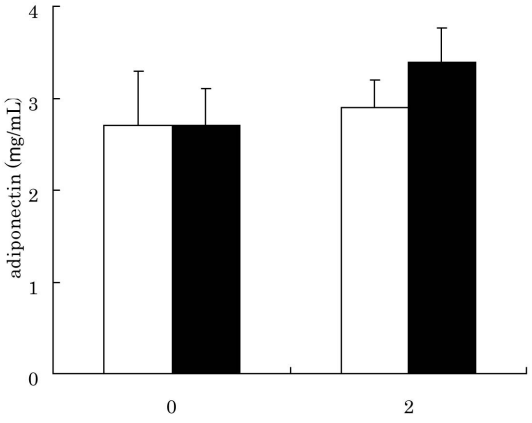

Figure 4. Effect of concentrated plum juice on plasma adiponectin concentrations in Wistar rats. Open bar, water-treated group; filled bar, plum juice-treated group. Values are means ± SE for eight animals per group. *P<0.05 vs. water-treated Wistar fatty rats (4). Original figure was courteously provided by Dr. Yamakawa.

Ⅳ. Multiple inhibitory effects on pandemic influenza A (HINI) virus by mumefural and its derivatives isolated from Japanese apricot fruit juice concentrate

The most rapidly spreading infectious disease is influenza caused by influenza A virus (17, 18), which is continuously undergoing rapid genetic changes. The easy loss of effectiveness of the previous vaccine and difficulty in updating a new effective vaccine in time if the next influenza pandemic has emerged, as a result of high mutation rate of the virus, indicate the need for anti-influenza agents for clinical management of influenza (19).

We found that the fruit-juice concentrate of Japanese apricot markedly inhibited the growth of influenza laboratory strains A/PR/8/34 (H1N1), A/Aichi/2/68 (H3N2) and A/Memphis/1/71 (H3N2) *in vitro* (20). We have isolated five components from the fruit-juice concentrate of Japanese apricot kindly provided by Nakano BC Co. Ltd., Wakayama, Japan, 5-(hydroxymethyl)-2-formylfuran (HMF), 1-[5-(2-formylfuryl)methyl]-dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate (mumefural, MF (Fig. 5)), 2-[5-(2-formylfuryl) methyl]dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate (MF`), 1-[5-(2-formylfuryl)methyl]hydrogen 1-hydroxyethane-1,2-dicarboxylate (MA1) and 2-[5-(2-formylfuryl)methyl]hydrogen 1-hydroxyethane-1,2-dicarboxylate (MA2), using the HMF purchased from Tokyo Chemical Industry Co. Ltd. and HMF derivatives (MF, MF `, MA1 and MA2) prepared from the fruit-juice concentrate and fructose-citric acid or fructose-malic acid reaction and characterized by 1H nuclear magnetic resonance (300.13 MHz, Bruker) as standards. All five compounds were investigated for their inhibitory activities against functions of the novel influenza 2009 (H1N1) pandemic virus hemagglutinin (HA) and neuraminidase (NA) acting as potential targets of several anti-viral agents due to their crucial roles in the influenza virus life cycle (21-24). Both HA and NA are spike glycoproteins present on the outer side of the lipid bilayer surrounding ribonucleoproteins of influenza virus. HA spikes initiate virus infection, and NA spikes release and spread the progeny viruses from infected cells to neighboring cells. Whereas an HA spike contains receptor binding sites attaching to sialyl sugar chain receptors on the target cells mediating invasion, an NA spike contains sialic acid (substrate)-binding pockets catalyzing the cleavage of linkage between a terminal sialic acid and the sub-terminal sugar (sialidase enzymatic activity) in glycoconjuates on the cell surface (25). Disruption of the balance between HA and NA activities in binding to and discharge from the host cell, respectively, by disturbance of HA or NA or both functions could

decrease virus replication.

A hemagglutination inhibition assay was performed by pre-incubation of various concentrations of an inhibitor with 4 hemagglutination unit (HAU) of the 2009 pandemic influenza virus at 4 ℃ for 1 hour before addition of 0.5% guinea pig erythrocytes and the inhibitory activities were determined after 2-h incubation at 4℃. As shown in Table 1, MF and MF` inhibited the viral hemagglutination at minimum hemagglutination concentrations of 3.1 and 6.3 mM, respectively.

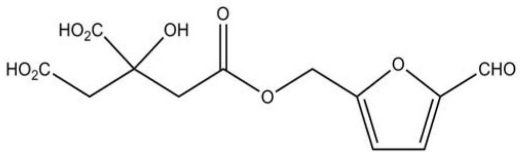

Figure 5. Chemical structure of mumefural (MF), 1-[5-(2-formylfuryl)dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate.

An inhibition study for sialidase activity of the NA spike was carried out at 37℃ by 15-min pre-incubation of an inhibitor-virus mixture, followed by a 15-min 2'-(4-methylumbelliferyl)-Neu5Ac (MU-Neu5Ac, fluorogenic substrate) hydrolysis reaction. The MU products released from MU-Neu5Ac were reduced depending on the concentration of the inhibitor as shown in Fig. 6 (In the absence of an inhibitor, the largest amount of MU released was considered as 100% sialidase activity). All HMF and HMF conjugated with citric (MF and MF`) or malic (MA1 and MA2) acids had viral sialidase inhibitory activity: MF was the most active anti-sialidase compound with an IC_{50} value of 0.21

Table 1. Inhibitory activities of mumefural and related compounds against hemagglutination and sialidase activities of a pandemic influenza A (A/Narita/1/2009 (H1N1)) clinical isolate

Compound	Hemagglutination activity, MC[a] (mM (mg/mL))	Hemagglutination inhibitory activity, MIC[b] (mM (mg/mL))	Sialidase inhibitory activity, IC50[c] (M)
HMF	0	0	$3.34 \pm 0.36 \times 10^{-2}$
MA1	6.3 (1.5)	UD	$1.64 \pm 0.31 \times 10^{-3}$
MA2	1.6 (0.4)	UD	$7.13 \pm 0.93 \times 10^{-4}$
MF	6.3 (1.9)	3.1 (0.9)	$2.07 \pm 0.11 \times 10^{-4}$
MF`	12.5 (3.8)	6.3 (1.9)	$1.62 \pm 0.22 \times 10^{-3}$
Fetuin	0	(1.3)	ND
DANA	ND	ND	$5.64 \pm 1.57 \times 10^{-6}$
Zanamivir	ND	ND	$4.94 \pm 1.42 \times 10^{-10}$
OC	ND	ND	$1.50 \pm 0.42 \times 10^{-9}$

[a]MC, minimum concentration that caused hemagglutination of guinea pig erythrocytes.
[b]MIC, minimum concentration that inhibited viral hemagglutination of guinea pig erythrocytes.
[c]IC_{50}, concentration that inhibited viral sialidase activity by 50% relative to compound-free control reactions. Each value is the mean ± standard error of the mean (SEM) of two independent assays each run in duplicate.
UD, undetectable
ND, not determined

± 0.01 mM, followed by MA2 (IC_{50}, 0.71 ± 0.09 mM), MA1 (IC_{50}, 1.64 ± 0.31 mM) and MF`(IC_{50}, 1.62 ± 0.22 mM), and HMF, as expected, inhibited sialidase activity at a much higher IC_{50} value of 33.40 ± 3.60 mM (Fig. 6 and Table 1).

MF, which is the most effective inhibitor against both hemagglutination and sialidase activities of each HA and NA spike of pandemic A/Narita/1/2009 (H1N1) strain, was used as a representative of HMF derivatives for testing its efficacy against this 2009 (H1N1) strain in cell culture. Whereas well-known anti-influenza compounds, OC and DANA, tested as positive controls in parallel showed IC_{50} values of 2.13 ± 1.38 nM and 10.9 ± 12.90 μM, respectively, MF inhibited 5 ± 3%, 10 ± 6% and 62 ± 3% of the replication of A/Narita/1/2009 (H1N1) at final concentrations of 5×10^{-9}, 5×10^{-6} and 5×10^{-3} M, respectively (Fig. 7).

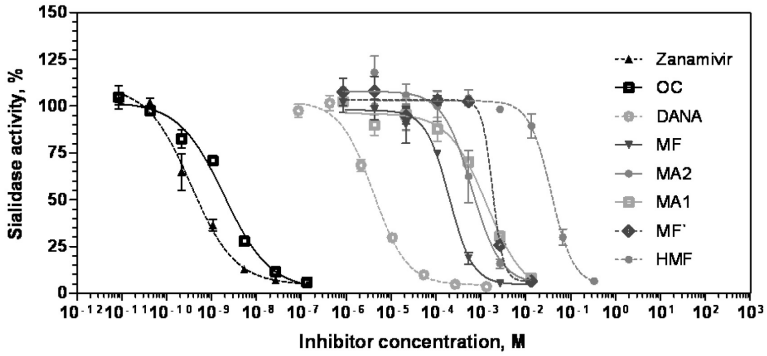

Figure 6. Dose-dependent inhibition of A/Narita/1/2009 (H1N1) sialidase activity of the neuraminidase spike by mumefural (MF) and related compounds. OC: osertamivir carboxylate; DANA: 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid

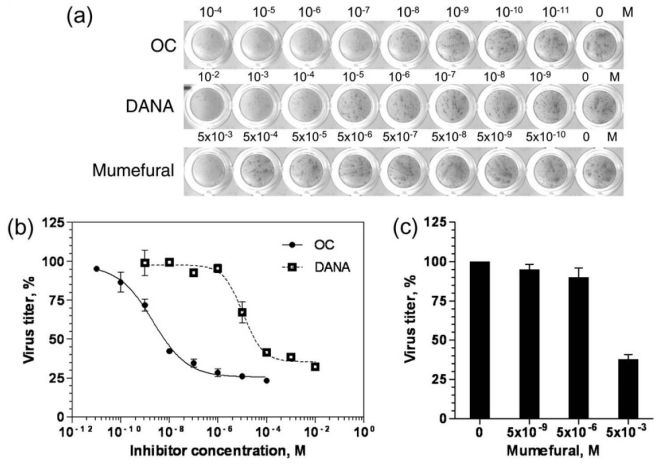

Figure 7. Decrease of A/Narita/1/2009 (H1N1) replication in MDCK-SIAT I cells treated with mumefural (MF) and standard agents, OC (osertamivir carboxylate) and DANA (2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid). (a) Colorimetric visualization of virus-infected cells in wells in the presence of the indicated inhibitor concentrations. The figures are representative of duplicate wells. (b) and (c) Fluorescence quantification of virus titers in infected cells, which were treated with serial dilutions of OC (filled circle, line graph), DANA (open square, line graph) or MF (bar graph).

Overall, our results suggest that the citric acid portion of HMF is required for hemagglutination inhibitory activity: unconjugated HMF is related to loss of the ability to inhibit viral hemagglutination. In comparison with interaction between sialic acid substrate and viral NA held firmly by hydrogen bonds between the carboxyl oxygen atoms of carboxylic acid on the pyranose ring of sialic acid with the NH groups of highly conserved Arg 292, Arg 371 and Arg 118 residues on one side of the active site of all influenza neuraminidases (26-29) (Fig. 8a), the HMF portion might provide a hydrogen bond acceptor (C=O of a carbonyl group in aldehyde on a furan ring) needed for its anti-NA function to make hydrogen bonding of O-H-N type with an influenza virus neuraminidase, whereas the linkage position of citric acid and malic acid to HMF producing different geometric isomers is likely to improve their sialidase inhibition potencies (Fig. 8b). Thus, the anti-influenza activity observed in cell culture by MF, a citric acid ester linked to HMF at the 1-position of the propane backbone, should be due to its multiple activities including anti-HA and anti-sialidase activities.

Currently, development of a multi-targeted monotherapeutic agent is highlighted as an effective approach to achieve high efficacy and delay development of target-based resistance in several fields including viruses (30-32). Further studies to examine the effects of various structural analogues of MF will be useful for finding the most suitable analogue against influenza viral replication.

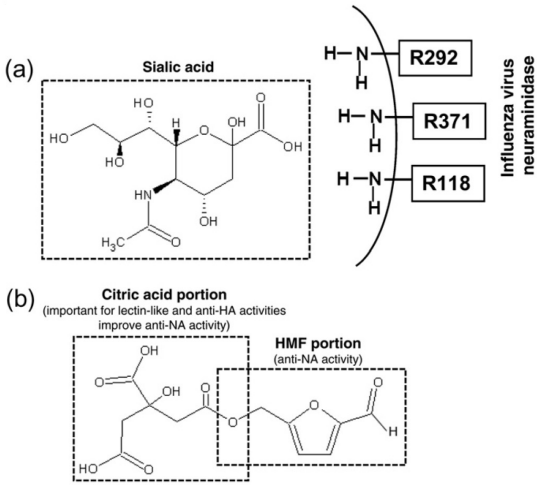

Figure 8. Crucial interaction between sialic acid substrate and the highly conserved residues, Arg 292, Arg 371 and Arg 118, in the active site of N1 neuraminidase (a), and proposed relationship between chemical structure and activities of generalized HMF derivatives (b).

V. Conclusions

Japanese apricot has several activities including inhibition of the motility of *H. pylori*, a major risk factor for stomach cancer, reduction of hyperglycemia in obese type 2 diabetic Wistar fatty rats, and multiple inhibitory effects on the growth of pandemic influenza A (H1N1) virus in cell culture. These activities increase the value of Japanese apricot in the food industry. Whereas isolated (+)-syringaresinol and mumefural have been shown to be anti-*H. pylori* and anti-influenza virus agents, respectively, it has not yet been revealed which components in Japanese apricot are responsible for the anti-hyperglycemic effects. More efforts for isolation, identification and characterization of active components and exploration of their biological activities to serve as promising leads for drug development should be useful for human therapy.

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