Mechanism-Based Inhibition of Recombinant Human Cytochrome P450 3A4 by Tomato Juice Extract

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This study investigates whether tomato juice can inhibit cytochrome P450 (CYP) 3A4-mediated drug metabolism. Three commercially available, additive-free tomato juices, along with homogenized fresh tomato, were analyzed for their ability to inhibit testosterone 6*β*-hydroxylation activity using human recombinant CYP3A4. Results were compared to that of grapefruit juice. Ethyl acetate extracts of the tomato juices moderately reduced residual activity of CYP3A4 testosterone 6*β*-hydroxylation activity by 19.3—26.2% with 0-min preincubation. Residual activity was strongly reduced by 69.9—83.5% at 20-min preincubation, a reduction similar to that of grapefruit juice extract, known to contain constituents of mechanism-based inhibitors. One juice extract (tomato juice C) showed irreversible dose- and preincubation time-dependent and partial nicotinamide adenine dinucleotide phosphate (NADPH)-dependent inhibition of CYP3A4 activity. Furthermore, we examined whether the CYP3A4 inhibitory effect of tomato juice was substrate dependent by examining midazolam 1′-hydroxylation activity and nifedipine oxidation activity, in addition to testosterone 6*β*-hydroxylation activity. Tomato juice showed a potent inhibitory effect on nifedipine oxidation activity, which was comparable to that on testosterone 6*β*-hydroxylation activity; however, it showed a weak inhibitory effect on midazolam 1′-hydroxylation activity. We conclude that tomato juice contains one or more mechanism-based and competitive inhibitor(s) of CYP3A4. Additionally, significant CYP3A4 inhibitory activity did not result from lycopene, a major compound in tomato. Although the active compound was uncertain, a strong CYP3A4 inhibitory activity was observed in other solanaceous plants, *i.e.*, potato, eggplant, sweet pepper, and capsicum. Therefore, responsible compounds in tomato are likely commonly shared among solanaceous vegetables.

Key words tomato juice; mechanism-based inhibition; food–drug interaction; human recombinant cytochrome P450 3A4

Many foods and/or beverages have recently been found to influence drug metabolism or transport, sometimes resulting in clinically important drug interactions. This food–drug interaction is a critical aspect of pharmacotherapy. Food–drug interaction can be viewed in terms of pharmacokinetics and pharmacodynamics. Pharmacokinetic interactions can involve enzymes and transporters that are implicated in drug absorption, distribution, metabolism, or excretion. Pharmacodynamic interactions involve the pharmacological effect of a drug or physiologic effect of a dietary constituent.[1)]

Foods that inhibit drug metabolism enzymes, such as cytochrome P450 (CYP), elevate the blood concentration of co-administered drugs, resulting in a food–drug interaction, which sometimes causes adverse effects.[2—4)] *In vitro* screening assays with beverages and foods such as beer, red wine, black and herbal tea, garlic, spices, mace, nutmeg, fruits, and fruit juices have all shown the ability to inhibit enzyme-mediated drug metabolism.[5—10)]

CYP3A4 is the most important drug metabolizing enzyme, in that it metabolizes more than 50% of all clinical drugs.[11)] It is expressed as the most abundant constituent in the human liver CYP enzyme system[12)] and is also expressed at substantial levels in the intestinal epithelial cells to metabolize and limit absorption of orally administered CYP3A4 substrate drugs.[13)] Grapefruit juice is an extensively studied dietary substance that is shown to irreversibly inhibit enteric CYP3A in a mechanism-based manner.[14—16)]

Mechanism-based inhibition (MBI) of CYP3A4 is characterized by nicotinamide adenine dinucleotide phosphate (NADPH)-, time-, and concentration-dependent enzyme inactivation that occurs when some substrates are converted by CYPs into reactive metabolites.[17)] Several phytochemicals, including GF-I-1 (4-[[6-hydroxy-7[[1-[(1-hydroxy-1-methyl)-ethyl]-4-methyl-6-(7-oxo-7*H*-furo[3,2-*g*][1]-benzopyran-4-yl)-4-hexenyl]oxy]-3,7-dimethyl-2-octenyl]oxy]-7*H*-furo[3,2-*g*][1]-benzopyran-7-one) and GF-I-4 (4-[[6-hydroxy-7[[4-methyl-1-(1-methylethenyl)-6-(7-oxo-7*H*-furo[3,2-*g*]-[1]benzopyran-4-yl)-4-hexenyl]oxy]-3,7-dimethyl-2-octenyl]oxy]-7*H*-furo[3,2-*g*][1]benzopyran-7-one) furanocumarins from grapefruit,[18)] rutaecarpine and limonene from *Evodia rutaecarpa*,[19)] methylenedioxyphenyl lignans from *Piper*,[20)] kaempferol from *Zingiber aromaticum*,[21)] 5-methoxypsoralen from *Foeniculum vulgare*,[22)] and lignans from *Phyllanthus amarus*,[23)] have all been shown to be responsible for MBI of CYP3A4. Moreover, interactions between these compounds and therapeutic drugs could occur *in vivo*.[24—26)]

Tomato juice is a very popular beverage, and epidemiological studies indicated that high consumption of tomato products is related to reduced risk of prostate cancer.[27,28)] Lycopene, a major ingredient in tomato, shows promising anticancer effects through its antioxidant activity, inhibition of cell cycle progression, apoptosis induction, increase in gap-junctional

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cell communication, inhibition of insulin-like growth factor I signal transduction, inhibition of androgen activation and signaling, *etc*.[29,30]

So far, there have been no reports suggesting an adverse food–drug interaction caused by the intake of tomato juice. However, in the process of screening a wide variety of common foods such as fruits, beverages, and health food products and herbal medicines, we have discovered the possibility that ethyl acetate extract of tomato juice inhibits recombinant human CYP3A4 activity *in vitro*. In this study, we attempted to demonstrate the ability of tomato juice to inhibit the catalytic activity of human recombinant CYP3A4 and the possibility of its influence on the disposition of drugs that are metabolized by CYP3A4.

MATERIALS AND METHODS

Materials Human recombinant CYP3A4 was purchased from Cypex Ltd., U.K. and stored at −80°C until use. NADPH, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Oriental Yeast, Ltd. (Tokyo, Japan). Testosterone and midazolam were purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). Nifedipine, 6β-hydroxyteststerone, nifedipine oxide, 1′-hydroxymidazolam, and lycopene were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other reagents were of HPLC or analytical grade, where appropriate.

Test Samples Additive-free tomato juices A, B, and C were purchased from Kagome Co., Ltd. (Mie, Japan), Nippon Del Monte Co. (Tokyo, Japan), and Ito En Ltd. (Tokyo, Japan), respectively. Fresh tomato (*Lycopersicon esculentum* Mill.) and grapefruit (*Citrus paradisi* Macf.) were obtained from local commercial sources. The minced fresh tomato (whole body) and grapefruit (without epicarp) were homogenized with an AM-8 homogenizer (Nihon Seiki Co., Ltd., Tokyo, Japan). Tomato juices, homogenized whole tomato, and grapefruit (1 mL) were each extracted with ethyl acetate (4 mL). The organic residues (3.5 mL) were dried, resuspended in 200 μL dimethyl sulfoxide (DMSO), and added to a reaction mixture (3.75 μL/250 μL) (described below). For concentration ratios of "2" and "4," the organic residues were resuspended in 100 and 50 μL DMSO, respectively. Potato (*Solanum tuberosum* L.), eggplant (*Solanum melongena* L.), sweet pepper (*Capsicum annuum* L. var. *grossum* Sendt.), and capsicum (*Capsicum annuum* L.) were dried at 50°C in an oven, crushed, and extracted with ethyl acetate (400 mg/mL). The organic residues were dried, resuspended in 200 μL DMSO, and added to the reaction mixture. Lycopen was dissolved in DMSO to a concentration of 500 μg/mL and added to the reaction mixture.

Measurement of CYP3A4 Activity CYP3A4 activity was measured by testosterone 6β-hydroxylation, nifedipine oxidation, and midazolam 1′-hydroxylation activity in human recombinant CYP3A4. The reaction mixture, containing 200 mM potassium phosphate buffer (pH 7.4), NADPH regenerating system (1.3 mM NADPH, 1.3 mM glucose-6-phosphate, 0.2 U/mL glucose-6-phosphate dehydrogenase, and 3.3 mM $MgCl_2$) along with 1.5% (3.75 μL) each of the lycopene or ethyl acetate extracts and the human recombinant CYP3A4 (16.5 pmol/mL), was preincubated at 37°C for 0, 2, 10, or 20 min. The reaction was started by the addition of 300 μM testosterone, 50 μM nifedipine, or 15 μM midazolam substrates. The substrate concentrations were approximately 4-fold of each K_m concentration. The K_m for each substrate was determined by linear regression from Lineweaver–Burk double reciprocal plots. The K_m values for testosterone, nifedipine, and midazolam were determined to be 73.3±14.0, 12.9±2.6, and 3.8±0.8 μM, respectively. These values were approximately similar to those in previous reports.[31,32] The final volume of the reaction mixture was 250 μL with a final DMSO concentration of 2%. The reaction was stopped by the addition of 500 μL ethyl acetate after 15 min. After centrifugation (15000×***g***, 5 min), 400 μL of supernatant was collected, dried, and resuspended in 200 μL of methanol. Analyses of the metabolites were performed by HPLC (SHIMADZU SCL-6B, C-R6A, SPD-6AV, LC-6A) equipped with ZORBAX SB-C8 (3.5 μm, 4.6×75 mm; Agilent Technologies, CA, U.S.A.). The mobile phase consisted of 10 mM ammonium acetate for solvent A and 10 mM ammonium acetate, 90% acetonitrile, and 10% methanol for solvent B. The metabolites were separated using a linear gradient method at a flow rate of 1.5 mL/min; for 6β-hydroxytestosterone, a linear gradient of 25—60% solvent B, 0—7 min was used; for 1-hydroxymidazolm, a linear gradient of 25—65% solvent B, 0—7 min was used; for nifedipine oxide, a linear gradient of 25—40% solvent B, 0—11 min was used. Quantification of the metabolites was performed by comparing the HPLC peak area at 245 nm to that of 11α-progesterone, the internal standard. The retention times for 6β-hydroxytestosterone, nifedipine oxide, 1′-hydroxymidazolam, and 11α-progesterone were about 3.8, 10.2, 5.0, and 6.7 min, respectively.

Preincubation Time- and NADPH-Dependent CYP3A4 Inactivation Assay The effect of preincubation time on CYP3A4 inactivation was determined by preincubating the tomato juice extract in a DMSO vehicle with human recombinant CYP3A4 in potassium phosphate buffer for 0, 10, or 20 min at 37°C in the presence of an NADPH regenerating system. The reaction was started by the addition of 300 μM testosterone as a substrate. Other conditions were same as mentioned above. The effect of NADPH on CYP3A4 inactivation was determined by preincubating tomato juice extract in a DMSO vehicle with human recombinant CYP3A4 in potassium phosphate buffer for 20 min at 37°C in the presence and absence of NADPH. The reaction was started by the addition of 300 μM testosterone and NADPH. Other conditions were same as mentioned above.

Effect of Ultrafiltration on CYP3A4 Inactivation Ultrafiltration studies were performed to determine whether the catalytic function of recombinant CYP3A4 could be restored following preincubation with tomato juice extract. Inactivation assays were performed by preincubating tomato juice extract in a DMSO vehicle with human recombinant CYP3A4 in potassium phosphate buffer for 20 min at 37°C in the presence of an NADPH regenerating system. The details have been described above except for the final reaction volume of 750 μL. The preincubated mixture was subsequently chilled on ice, transferred to Ultracel YM-30 filters (30000 nominal molecular weight limit regenerated cellulose membrane; Millipore, Yonezawa, Japan), and centrifuged at 5000×***g*** for 15 min. The retentate was washed with addition of 200 μL of potassium phosphate buffer (200 mM, pH 7.4), recentrifuged at 5000×***g*** for 30 min, and then resuspended with 200 μL of potassium phosphate buffer (200 mM, pH 7.4). A 80-μL aliquot was removed to determine the rate of 6β-hydroxytestosterone

Table 1. Effects of Ethyl Acetate Extracts of Tomato Juice, Grapefruit Juice, and Hypericin on CYP3A4 Activity

Specimens	Residual activity (% of control)		
	0 min	10 min	20 min
Testosterone 6β-hydroxylation			
Grapefruit juice (1)	37.9±1.8 (100)	29.4±1.8 (77.6)	14.4±1.8 (38.0)
Grapefruit juice (1/2)	63.3±2.0 (100)	42.5±2.0 (67.1)	36.5±2.0 (57.7)
Grapefruit juice (1/4)	80.0±1.4 (100)	52.8±1.4 (66.0)	45.9±1.4 (57.4)
Tomato juice A	78.8±0.1 (100)	42.7±0.1 (54.2)	29.8±0.1 (37.8)
Tomato juice B	80.7±1.7 (100)	37.1±1.7 (46.0)	25.8±1.7 (32.0)
Tomato juice C	73.8±3.1 (100)	32.7±3.1 (44.3)	16.5±3.1 (22.4)
Squeezed whole tomato	76.7±3.9 (100)	43.5±3.9 (56.7)	30.1±3.9 (39.2)
Hypericin	76.9±10.8 (100)	—	76.5±6.8 (99.5)
Nifedipine oxidation			
Tomato juice C	33.8±5.6 (100)	10.0±5.3 (29.6)	19.6±14.8 (58.0)
Midazolam 1′-hydroxylation			
Tomato juice C	70.5±7.0 (100)	44.0±23.2 (62.4)	37.0±8.7 (37.6)

( ): % of 0 min. Values are presented as mean±S.D. of three independent experiments. The stock solution concentration of a grapefruit juice extract, described in the Materials and Methods, is equivalent to a concentration ratio of "1."

formation. Control measurements were performed in the absence of tomato juice extract.

Statistical Analysis Statistical differences between the treatment and control groups were evaluated using Dunnett's test, and a p value less than 0.05 was considered significant.

RESULTS

This study focused on the CYP3A4 inhibitory activity of tomato juice. The preincubation time-dependent CYP3A4 inhibitory activity of three different commercially available tomato juices and homogenized fresh tomato was compared with grapefruit juice (Table 1). Grapefruit juice showed concentration- and preincubation time-dependent inhibition of CYP3A4. Similarly, all tomato juices or homogenized fresh tomato tested showed preincubation time-dependent inhibition of CYP3A4. In the 0-min preincubation, tomato juices A, B, C and homogenized fresh tomato were shown to inhibit CYP3A4 activity, comparable to that of quarter and half strength grapefruit juice, respectively. However, tomato juice C exhibited potent CYP3A4 inhibitory activity comparable to that of a full-strength concentration of grapefruit juice (85.6 *vs.* 83.5% inhibition) for the 20-min preincubation. In addition, hypericin, known as a reversible inhibitor[33] of CYP3A4 and a constituent of St. John's wort (*Hypericum perforatum*), did not enhance the inhibitory activity for prolonging preincubation. We also examined whether tomato juice C demonstrated an inhibitory effect against midazolam 1′-hydroxylation activity and nifedipine oxidation activity. The inhibitory effect of tomato juice C against nifedipine oxidation was the same as testosterone at 20-min preincubation (80.4 *vs.* 83.5% inhibition), but showed stronger inhibition than testosterone at 0-min preincubation (66.2 *vs.* 26.2% inhibition). On the other hand, although the inhibitory effect against midazolam 1′-hydroxylation activity was comparable to that of testosterone at 0-min preincubation (29.5% inhibition), it showed weaker inhibition at 20-min preincubation (63.0% inhibition).

Furthermore, we examined the dose-dependent inhibition of tomato juice C against CYP3A4 activity for preincubation at 0 and 20 min (Fig. 1). Although both 0- and 20-min preincubation showed dose-dependent inhibition of CYP3A4 activity,

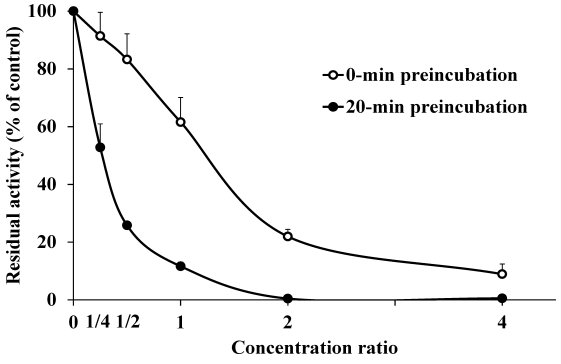

Fig. 1. Preincubation Time- and Dose-Dependent Inhibition of Tomato Juice C on Human Recombinant CYP3A4 Activity

Values are presented as mean±S.D. of three independent experiments. The stock solution concentration of a tomato juice C extract, described in the Materials and Methods, is equivalent to a concentration ratio of "1."

the 20-min preincubation showed more potent inhibition of CYP3A4 activity than the 0-min preincubation.

To examine whether the components of tomato juice inhibited human recombinant CYP3A4 in a mechanism-based manner, we used conditions in the presence or absence of an NADPH-regenerating system during preincubation followed by ultrafiltration. Results are shown in Table 2. Results showed that in the presence of NADPH, tomato juice C extract inhibited CYP3A4 catalytic activity by 93.3%, whereas in the absence of NADPH, tomato juice C inhibited activity by only 63%. Furthermore, recombinant CYP3A4 activity was not restored by ultrafiltration following preincubation with the tomato juice C extract.

The product description for tomato juice C stated that the lycopene concentration was approximately 110 μg/mL of juice. Therefore, we evaluated the inhibitory activity of lycopene in corresponding concentrations (approximately 7.5 μg/mL of reaction mixture, which is equivalent to a concentration ratio of "1" in Fig. 2). The inhibitory activity of lycopene in 20-min preincubation was weak with an inhibition of only 28%, though that concentration corresponds to twice the lycopene contained in the tomato juice C extract that showed more than 80% inhibition (Fig. 2).

Table 2. Effect of NADPH and Ultrafiltration on Inactivation of Testosterone 6β-Hydroxylation Activity of Human Recombinant CYP3A4

Specimens	Residual activity (% of control)		
	Preincubation 20 min		
	NADPH (−)	NADPH (+)	NADPH (+)+ultrafiltration
Control	100	100	100
Tomato juice C	37.0±13.1	6.7±2.7	3.8±2.1

Values are presented as mean±S.D. of three independent experiments.

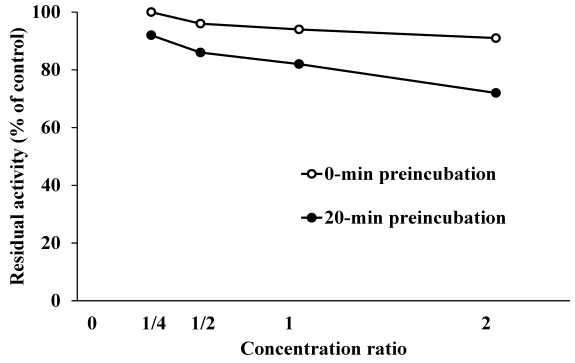

Fig. 2. Effect of Lycopene on Testosterone 6β-Hydroxylation Activity of Human Recombinant CYP3A4

Values are presented as the mean of duplicate experiments. The concentration ratio of "1" is equivalent to approximately 7.5 μg/mL of lycopene in the reaction mixture, corresponding to tomato juice C.

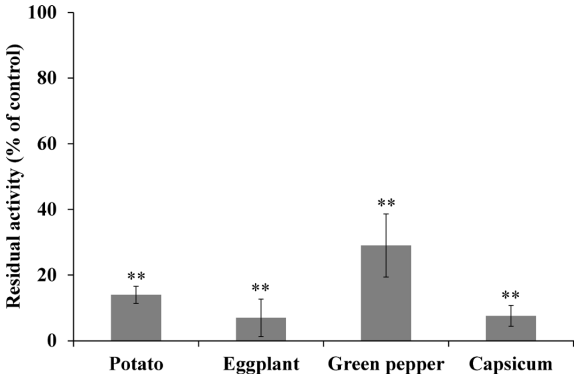

Fig. 3. Inhibitory Effects of Edible Solanaceous Plants on Testosterone 6β-Hydroxylation Activity of Human Recombinant CYP3A4

Experimental conditions are described in the Materials and Methods except that preincubation is performed for 2 min. Each value is mean±S.D. **$p<0.01$ compared to control (n=3).

Subsequently, we attempted to evaluate the CYP3A4 inhibition ability of other edible solanaceous plants, *i.e.*, potato, eggplant, sweet pepper, and capsicum. All samples tested showed potent inhibition of testosterone 6β-hydroxylation activity that is mediated by human recombinant CYP3A4 (Fig. 3).

DISCUSSION

This study demonstrates that an ethyl acetate extract of tomato juice phytochemicals strongly inhibited CYP3A4-mediated testosterone 6β-hydroxylation activity in a magnitude comparable to that of grapefruit juice through MBI, and may be in part by competitive inhibition. The reasons for which CYP3A4 inhibitory activity of tomato juice is regarded as MBI are as follows: (1) The inhibitory effect of tomato juice on CYP3A4 catalytic activity was dependent on the preincubation time (Table 1, Fig. 1). This approach is methodologically correct because hypericin, a reversible inhibitor of CYP3A4 contained in St. John's wort,[33] did not show preincubation-dependent inactivation (Table 1). (2) The inhibition partially depended on the presence of an NADPH regeneration system during preincubation (Table 2). In this regard, because an inhibition of 63% was observed in the absence of NADPH, the inhibition of CYP3A4 with tomato juice cannot be fully explained by a mechanism-based model. Therefore, it is thought that tomato ingredients inducing MBI coexist with those inducing reversible inhibition. (3) CYP3A4 catalytic activity from 20-min preincubation was not restored after ultrafiltration (Table 2).

Previously, it was reported that the different tomato juice product from products used in this study did not demonstrate an inhibition on human microsomal CYP3A activity, as evaluated by midazolam 1′-hydroxylation activity.[34] However, we identified that tomato juice strongly reduced CYP3A4 activity through MBI. The differences in CYP3A4 source, *i.e.*, microsomes *vs.* recombinant CYP3A4, tomato juice product, and/or preincubation times (5 *vs.* 20 min) that were used in this study are likely the cause of this discrepancy. Besides, the effects of tomato juice on midazolam metabolism were smaller than those on testosterone and nifedipine in a substrate-dependent inhibitory activity study (Table 1). In other words, it is expected that the sensitivity of evaluated CYP3A4 inhibition for the prediction of potential interactions differs between conditions for enzyme source, selected substrate, source of test sample, and preincubation or incubation time. Regardless, our experiment demonstrated that all three commercial tomato juices and homogenized fresh tomato showed potent inhibitory activity against CYP3A4-mediated testosterone 6β-hydroxylation, according to results for prolonged preincubation.

In this study, tomato juice showed a potent inhibitory effect against nifedipine oxidation activity, comparable to that of testosterone 6β-hydroxylation activity, but a weaker inhibitory effect against midazolam 1′-hydroxylation activity. As for substrate used, the substrate-dependent phenomena and atypical kinetics of CYP3A4-mediated drug–drug interactions were documented in several previous *in vitro* studies, which indicated the existence of distinct and preferential binding domains for each substrate subgroup, namely, midazolam, testosterone, and nifedipine.[32,35,36] Furthermore, the inconsistent and unclear effects of ginsenoside, an active ginseng compound, on human drug-metabolizing enzymes could be explained by substrate-dependent phenomena.[37] Although the difference in the inhibitory effect was shown with various substrates, we concluded that this CYP3A4 inhibition occurs

in common to tomato. Accordingly, in order to accurately assess the CYP3A4-mediated interaction potential, different classes of CYP3A4 probe substrates must be used to minimize the oversight that could occur from the use of only one class of CYP3A4 probe substrates.

At present, ingredients responsible for MBI are uncertain. However, it has been recently reported that several flavonoids, such as kaempferol, quercetin, naringenin, apigenin, myricetin, chrysin, diosmetin, and luteolin, inhibit the catalytic activity of CYP3A4.[38—40] Furthermore, naringenin, apigenin, luteolin, kaempferol, myricetin, and quercetin are the major flavonoid components found in tomato.[41] Flavonoids, which are also major constituents of grapefruit juice, are not known to be mechanism-based inhibitors. Naringin, the major flavonoid present in grapefruit juice, and quercetin do not reproduce the grapefruit juice effect when administered orally, suggesting that flavonoids are not the active compounds.[42—44] Therefore, these flavonoids are reversible inhibitors and do not seem to be irreversible mechanism-based inhibitors.

We attempted to verify that a CYP3A4 inhibitory effect was achieved with lycopene, a major compound in tomato, that gained attention for its ability to reduce the incidence of prostate cancer. As a result, no marked CYP3A4 inhibitory activity was shown, and lycopene does not seem to be related to CYP3A4 inhibition (Fig. 2). However, other solanaceous plants, such as potato, eggplant, sweet pepper, and capsicum, strongly inhibited CYP3A4-mediated testosterone 6β-hydroxylation (Fig. 3). From this result, other solanaceous plant extracts appear to have a stronger inhibitory effect than tomato juice extracts, but direct comparison of the inhibitory activity is not possible because they were extracted from dry matter. However, the CYP3A4 inhibitory effect was observed in all solanaceous plants examined. Therefore, responsible compounds found in tomato seem to be shared with solanaceous vegetables. Further investigation to identify specific CYP3A4-inhibiting compounds is necessary.

Tomato juice is a very popular beverage and is likely often consumed together with medicine. Additionally, because epidemiological studies indicated that a high consumption of tomato products is related to reduced incidence of prostate cancer,[27,28] an individual may consume it in large amounts. When irreversible strong enzyme inhibition occurs with this generally consumed beverage, it may gain the same type of attention currently given to grapefruit.

Grapefruit juice is the most commonly known food considered in food–drug interaction, and it is able to markedly elevate the oral bioavailability of several CYP3A4 substrate drugs, including several calcium antagonists, immunosuppressive agent cyclosporine, and some antilipemic agents. The predominant mechanism for this interaction is the inhibition of CYP3A4 in the small intestine, resulting in a significant reduction of drug presystemic metabolism. Hepatic CYP3A4 activity seems to be unaffected by grapefruit juice when consumed in usual volumes. In the case of tomato juice, although ingredient characteristics were unclear, if the active compounds are easily absorbed from the gastrointestinal tract, not only intestinal CYP3A4 but also hepatic CYP3A4 may be inhibited by the responsible ingredients of tomato. Accordingly, not only the elevation in the bioavailability of CYP3A4 substrate drugs but also a decrement in drug clearance could possibly lead to more serious drug interaction.

In conclusion, we have found the possibility that an ethyl acetate extract of tomato juice inhibits CYP3A4 activity *via* MBI. So far, there has been no clinical report suggesting an adverse food–drug interaction caused by the intake of tomato juice. This finding is important and suggests that tomato juice dramatically influences the disposition of drugs metabolized by CYP3A4 similar to grapefruit juice. Accordingly, the possibility of an adverse food–drug interaction, caused by the impact of tomato juice on CYP3A4 metabolism, should be examined *in vivo*.

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