The effect of lycopene on the total cytochrome P450, CYP1A2 and CYP2E1

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Abstrak

Tujuan *Beberapa penelitian terakhir melaporkan bahwa karotenoid memiliki kemampuan untuk memodulasi sistem metabolisme xenobiotik. Penelitian ini dirancang untuk menyelidiki pengaruh likopen suatu karotenoid non-provitamin A terhadap enzim sitokrom P450 total, CYP1A2 dan CYP2E1 mikrosom hati tikus.*

Metode *Fraksi mikrosom diperoleh dengan metode sentrifugasi diferensial yang dikombinasi dengan agregasi mikrosom menggunakan ion kalsium. Kadar sitokrom P450 total, aktivitas CYP1A2 (asetanilid-4-hidroksilase) dan CYP2E1 (p-nitrofenol hidroksilase) dipelajari pada mikrosom hati tikus Sprague-Dawley jantan. Hewan coba diberi likopen dalam dosis 0 mg/kgBB/hari, 25 mg/kgBB/hari, 50 mg/kgBB/hari atau 100 mg/kgBB/hari per oral selama 14 hari. Data dianalisis dengan uji ANOVA.*

Hasil *Kadar sitorom P450 total dan aktivitas asetanilid-4-hidroksilase tidak dipengaruhi oleh semua perlakuan. Aktivitas CYP2E1 menurun bermakna oleh pemberian likopen 100 mg/kgBB/hari selama 14 hari (7,88 ± 2,04 vs 12,26 ± 2,77 n mol/min/mg prot).*

Kesimpulan *Hasil penelitian ini menunjukkan bahwa likopen tidak mempengaruhi sitokrom P450 total dan aktivitas CYP1A2 (asetanilid 4-hidroksilase), namun memiliki efek inhibisi pada aktivitas CYP2E1 (p-nitrofenol hidroksilase).* ***(Med J Indones 2009; 18: 233-8)***

Abstract

Aim Some carotenoids such as canthaxantin, astaxanthin and beta apo-8'-carotenal were reported to have modulatory effect on the cytochrome P450. The present study was conducted to investigate the effects of lycopene, a non-provitamin A carotenoid, on microsomal cytochrome P450, CYP1A2 and CYP2E1.

Methods Total cytochrome P450 levels, CYP1A2 and CYP2E1-catalyzed reactions (acetanilide 4-hydroxylation and p-nitrophenol hydroxylation) were studied in the liver microsomes of male Sprague Dawley rats. Microsomes were prepared using differential centrifugation combined with calcium aggregation method. Lycopene was orally administered in the dosages of 0, 25, 50 or 100 mg/kgBW/day for 14 days in a repeated fashion. Data were analyzed using ANOVA test.

Results Total cytochrome P450 level and acetanilide 4-hydroxylase activity were unaffected by any of the treatments. The CYP2E1 probe enzyme (p-nitrophenol hydroxylase) was significantly reduced by repeated administration of 100 mg/kgBW/day lycopene (7.88 ± 2.04 vs 12.26 ± 2.77 n mol/min/mg prot).

Conclusion The present results suggest that lycopene does not affect the total cytochrome P450 or CYP1A2 activity but it inhibits the activity of CYP2E1 (p-nitrophenol hydroxylase) in the rat. ***(Med J Indones 2009; 18: 233-8)***

Key words: *lycopene, cytochrome P450, CYP1A2, CYP2E1*

Cytochrome P450 enzymes play a very important role in phase I metabolism of many xenobiotic substrates such as drugs, carcinogens, food additives, pollutants, pesticides, environmental chemicals and endogenous substances. Most of the xenobiotics biotransformation will produce inactive metabolites, but some of the process can also produce active products. Mostly the metabolites are non-toxic, but some of the phase I enzymes mediated reactions metabolize procarcinogens to biologically active carcinogens.[1]

In recent years, many experimental evidences have suggested that carotenoids have the ability to alter xenobiotic-metabolizing enzymes.[2,3] Several carotenoids, especially β-carotene, have received tremendous research interest because they are found in high concentration in human blood.[4] Epidemiological studies showed that the consumption of carotenoids may help prevent cancer and heart disease in humans. However, high intake of these micronutrients failed to reduce tumor incidence, and two intervention trials (ATBC and CARET) revealed that long-term β-carotene supplementation can actually increased the relative risk for lung cancer among heavy smokers and asbestos workers.[5,6] It was suggested that the high relative lung cancer risk recorded in the two

intervention trials might be linked to phase I induction of drug metabolism. Paolini et al. reported that beta carotene supplementation can induce the activities of certain isoforms of cytochrome P450; namely CYP1A1/2. CYP3A1/2, CYP2E1, CYP2B1/2.[7]

Other carotenoids that have inducing effects on xenobiotic-metabolizing enzymes are canthaxanthin, astaxanthin and beta apo-8'-carotenal. On the other hand, lycopene, was shown to have inhibitory effect *in vitro* on CYP2E1, an isoform of cytochrome P450, which is responsible for the conversion of certain xenobiotics to carcinogens.[7,8]

Lycopene is a natural pigment synthesized by plants and microorganisms but not by animals. It is a carotenoid, an acyclic isomer of β-carotene, and has no vitamin A activity (Figure 1).[8] Lycopene has been the subject of intense study with respect to its antioxidant activity and potential in preventing chronic diseases such as certain cancers and coronary heart disease.[9]

This study was designed to investigate whether lycopene can affect the microsomal total cytochrome P450 level, activities of CYP1A2 and CYP2E1 in male rats. CYP1A2 and CYP2E1 are two of several isoforms of cytochrome P450, which were known to be important in the metabolism of toxicants and carcinogens.

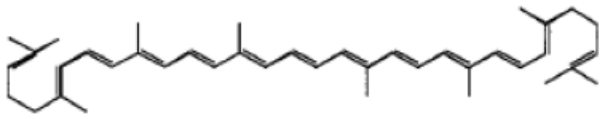

Figure 1. Chemical structure of lycopene

METHODS

Material and methods

Chemicals: Lycopene 5,6% (RocheAG); Carboxymethyl cellulose, sucrose, $CaCl_2$, $MgCl_2$, H_2SO_4, KH_2PO_4, K_2HPO_4, sodium dithionite, KCl, Na_2CO_3 sodium dodecyl sulphate, sodium bisulfite, sodium sulfite, EDTA, phenol, NaOH, NaN_3, 1-aminonaphtol-4-sulfonic acid, Folin Ciocalteu's reagent, maleic acid, ascorbic acid (E.Merck); $CuSO_4$, Tris-HCl, glucose 6-phosphate.sodium salt, trichloroacetid acid, bovine serum albumine fraction V, ammonium molybdate, 4-hydroxyacetanilide, $NADP^+$, glucose 6-phosphate dehydrogenase (Sigma). K-Na-tartrate, acetanilide (Fluka); p-nitrofenol (Aldrich); CO(g).

This is an experimental study to investigate the effects of lycopene on microsomal total cytochrome P450, CYP1A2 and CYP2E1, which was conducted by *ex vivo* procedures in male rats (lycopene was given to the animals *in vivo*, followed by evaluation of microsomal enzyme activities).

Twenty for male Sprague-Dawley rats weighing ± 200g were randomly allocated to four groups of six rats (designated to receive lycopene 25 mg/kgBB/day, 50 mg/kgBB/day, 100 mg/kgBB/day or control daily for 14 consecutive days). They were fed on rat pellets and tap water *ad libitum*, throughout the experiment. The care of the animals was complied to the institutional guides for use of laboratory animals. Controls (0 mg/kgBB/day) received vehicle only. At the end of the experiments, the rats were sacrificed, and the livers were rapidly removed and processed separately for microsomal isolation.

Isolation of microsome

Rat liver microsome were isolated according to method of differential centrifugation and microsomal aggregation with calcium as described previously.[10]

Enzyme assays

The protein contents of liver homogenate and microsomal fractions were determined according to the method of Lowry et al., (modified by Paterson), using bovine serum albumin as standard.[11]

The total cytochrome P450 was determined as described previously by Omura & Sato, 1964.[12] The activity of glucose-6-phosphatase was determined as marker for the microsomal fraction.[13] Activities of acetanilid-4-hydroxylase and p-nitrophenol hydroxylase were used as probe enzyme of CYP1A2 and CYP2E1, respectively.[14,15]

Statistical analysis

The degree of normality and variant homogeneity of the data were tested using Kolmogorov-Smirnov (K-S test for rankable scores) and F test. Since the data obtained showed a normal and homogeneous distribution, they were analyzed using the related one-way ANOVA followed by Tukey method at a significance level of $\alpha = 0.05$. Results are expressed as means ± S.D.

RESULTS

Protein content of homogenate and extent of purification of the isolated microsomal preparation

The microsomal preparation was homogenously purified as shown by the high relative specific activity (RSA) of glucose-6-phosphatase of 72.32 ± 17.53 (Table 1).

Total cytochrome P450, specific activity of acetanilide-4-hydroxylase and p-nitrophenol hydroxylase

The level of microsomal cytochrome P450 specific activity of acetanilide-4-hydroxylase (CYP1A2) and p-nitrophenol hydroxylase (CYP2E1) was provided in Table 2 and the spectrum of absorption was shown in Figure 2. There was no significant difference in the total cytochrome P450 among the 4 treatments (p>0.05) as it was also shown in the spectrum of absorption.

Acetanilide-4-hydroxylase is a probe enzyme used in the current study to determine the activity of CYP1A2 in the microsomal fraction. The result of the assay was provided in Table 4. It was shown that there's no significant difference in the 4 treatment groups (p>0.05). The treatment of the experimental animal with lycopene 25 mg/kgBW/day, 50 mg/kgBW/day and 100 mg/kgBW/day did not alter the activity of acetanilide 4-hydroxylase (CYP1A2) rat liver microsomes.

The hydroxylation of p-nitrophenol is a probe reaction used to determine the activity of CYP2E1. Assay results is showed in Table 5. Lycopene 100 mg/kgBW/day administered for 14-days reduced the activity of the enzyme 35.73% (p=0.002) (Tabel 5, Tabel 6). Lycopene 25 mg/kgBW/day and 50 mg/kgBW/day administered for 14-days also resulted in the reduction in the activity of the enzyme (15.99% and 17.86%, respectively), but not significantly different from control (p>0,05)

Table 1. Glucose-6-phosphatase specific activity (SA) and relative specific activity (RSA)

Fraction	Protein		Glucose-6-phosphatase	
	mg/g liver	% homogenate	SA (± SD) (μmol Pi/min/mg protein)	RSA*)
Homogenate (n=24)	324.11 ± 46.21	100	0.031 ± 0,007	
Microsome (n=24)	24.46 ± 4.22	7.54	0.162 ± 0,038	72.32 ± 17.53

*) RSA was defined as the percentage of total homogenate activity present in microsomal fraction divided by the percentage of total protein present in that fraction.

Table 2. Total cytochrome P450, specific activity of CYP1A2 and CYP 2E1

	CONTROL	L-25	L-50	L-100	P
Total cyt P450 (nmol/mg prot)	0.73 ± 0.21	0.62 ± 0.18	0.58 ± 0.17	0.64 ± 0.18	0.551
Specific activity of CYP1A2 (nmol/min./mg prot)	11.18 ± 2.37	10.11 ± 2.03	12.65 ± 1.40	10.31 ± 1.67	0.121
Specific activity of CYP2E1 (nmol/min./mg prot)	12.26 ± 2.77	10.30 ± 1.90	10.07 ± 2.69	7.88 ± 2.04*	0.038

Data are expressed as mean ± SD.

L-25: Lycopene 25mg/kgBW/day, L-50: Lycopene 50mg/kgBW/day, L-100: Lycopene100 mg/kgBW/day

*: p=0.02 vs control group

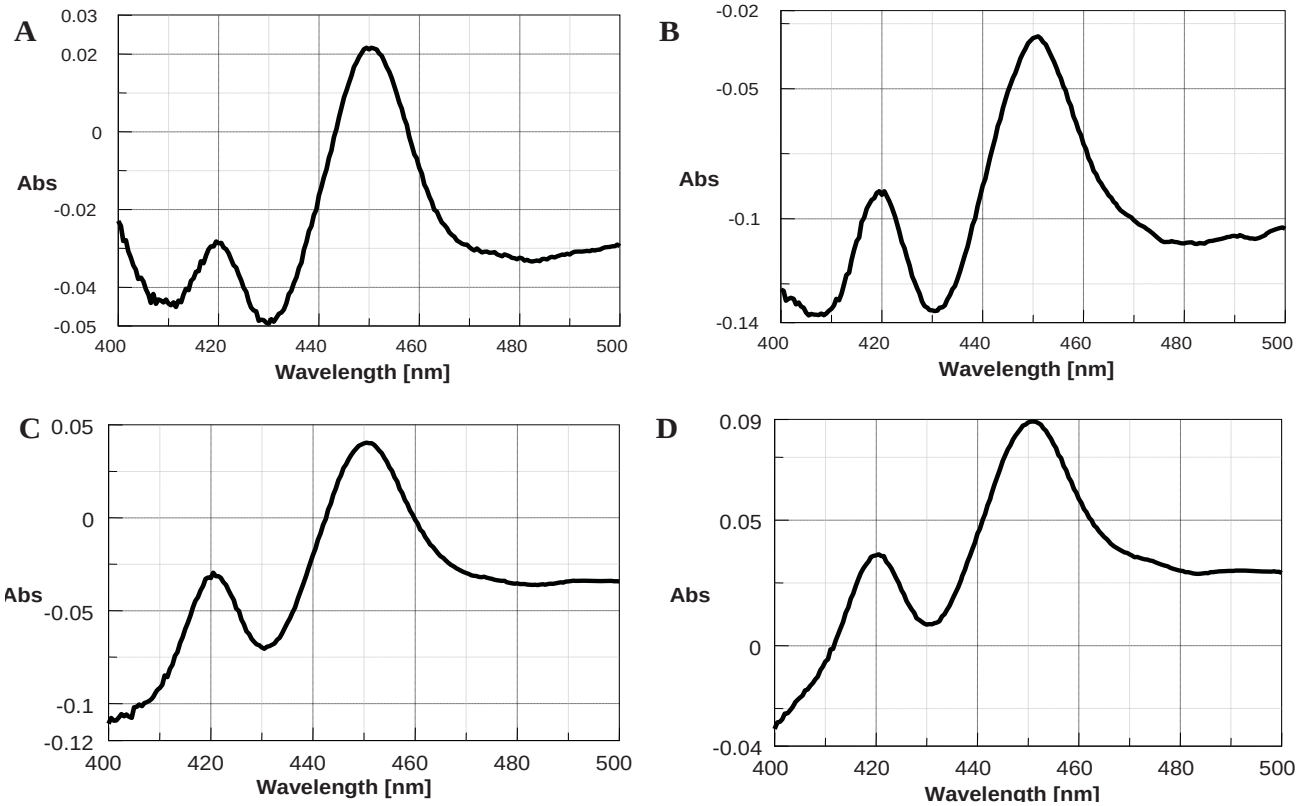

Figure 2. Absorption spectrum of CO-reduced microsomal fraction

No significant difference of absorption spectrum among the 4 groups of treatments
(A: control, B: lycopene 25mg/kgBW/day, C: 50 mg/kgBW/day, D: 100 mg/kgBW/day).

DISCUSSION

Lycopene is a non-provitamin A carotenoids, has been the subject of intense study with respect to its antioxidant and antiproliferative effects. Data from epidemiological studies showed an inverse correlation between the intake of tomato and tomato products rich in lycopene and the incidence of several diseases such as cardiovascular and some types of cancer.[6]

Some recent evidences showed that carotenoids have the ability to modulate the xenobiotics-metabolizing enzymes.[2,3] These findings were triggered by CARET (Carotene and retinol efficacy trial) and ATBC (The alpha tocopherol, beta carotene cancer prevention study), which reported that high dose beta-carotene supplementation in the smokers increased the risk of lung cancer.[5,6] It was suggested that the high relative lung cancer risk recorded in the two intervention trials might be linked to phase I induction of drug metabolism.[7]

We report here the effects of repeated lycopene supplementation on the total cytochrome P450, CYP1A2 and CYP2E1. Both enzymes (CYP1A2 and CYP2E1) are two of some isoforms of CYP which are known to activate toxicants and carcinogens (CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2E1).[16] CYP1A2 is the isoform of cytochrome P450 converts polycyclic aromatic hydrocarbon (PAH) to an epoxide form and acts in the oxidation reaction of heterocyclic amines. Increased activity of CYP1A2 is an indicator of the increased risk to carcinogenesis.[16] CYP2E1 is an inducible enzyme that is involved in the metabolism of ethanol, paracetamol, N-nitrosamine and many organic solvents.[17] Increased activity of CYP2E1 is likely to cause toxicities related to the activation of procarcinogen to carcinogen, formation of toxic reactive metabolites from acetaminophen and also caused increased production of free radicals (ROS).[18]

Total cytochrome P450 and the activity of acetanilide-4-hydroxylase (CYP1A2) did not showed any significant

difference ($p>0.05$) between groups. It showed that lycopene administered repeatedly for 14 days up to 100 mg/kgBB did not alter the total cytochrome P450 and the activity of CYP1A2 rat microsomal fraction.

Data obtained from the present study complements data from other studies using carotenoids. Canthaxanthin, asthaxantin (non-provitamin A carotenoids) and β-apo-8'-carotenal were shown to be strong inducer of CYP1A1 and CYP1A2, but only canthaxanthin induced the total cytochrome P450.[19]

Gradelet et al. reported that 300 mg/kgBW beta-carotene supplementation did not induce CYP1A1/2,[3] while Paolini et al. reported that beta carotene given in a dose of 500 mg/kgBW induced CYP1A2.[7] The difference between the two results was estimated because of the differences in dose administration. Thus far, the results with beta-carotene are still controversial and inconclusive.

Nagao hypothesized that the difference in the potency of carotenoids induction in the cytochrome P450 did not lied in the level of provitamin A activity, but in the oxidative metabolism of each carotenoids that binds to Ah receptor. Beta-apo-8'-carotenal, is one of the metabolites produced from the oxidative metabolism of β-carotene; while 4-oxo-retinoic acid is a metabolite produced by autooxidation of canthaxanthin. Both metabolites have the ability to induce cytochrome P450, that oxidize carcinogens and increase the catabolism of retinoic acid.[20]

In contrast, metabolite produced by oxidative metabolism of lycopene, 2,6-cyclolycopene-1,5-diol maintain the carbon backbone of its parent compound. It was not known whether 2,6-cyclolycopene-diol have the ability to catabolize retinoic acid. It was assumed that the effect will be different from β-Apo-8'-carotenal and 4-oxo-retinoic acid.[20]

Determination of p-nitrophenol hydroxylase (CYP2E1) in the present study showed that lycopene 100 mg/kgBB reduced the activity of CYP2E1 significantly versus control (Δ = 35,73%, $p<0.05$). The reduction of the CYP2E1 activity is also shown at 25 mg/kgBW and 50 mg/kgBW lycopene (15,98% and 17,86%, respectively), but not statistically significant. There was a tendency of dose dependency in the inhibition of p-nitrophenol hydroxylase activity.

The result was similar to the *in vitro* study by Xu et al. who investigated the apoptosis effect of lycopene in HepG2 cells expressing CYP2E1.[21,22] It was reported that lycopene inhibits the apoptosis and alcohol-induced oxidative stress. The same research group reported that lycopene 10 μM reduced the toxicity of arachidonic acid in cell culture expressing CYP2E1. Study by Astorg et al. showed that lycopene administered in diet reduced the initiation of liver preneoplastic foci induced by diethylnitrosamine in the rats.[23] It was reported that the this protective effect was not from its antioxidant effect but from the inhibitory effect of lycopene to metabolize diethylnitrosamine, through CYP2E1. Diethylnitrosamine has to be metabolised by CYP2E1 to nitrosamine to become carcinogenic.

Kim et al. also confirmed Astorg's work which reported that lycopene has the chemopreventive effect of pulmonary cancer induced by diethylnitrosamine (DEN), N-methyl-N-nitrosourea (MNU) and 1,2-dimethylhydrazine (DMH).[24]

CYP2E1 is an enzyme that has the capacity to metabolize xenobiotics. Organic solvents (bromobenzene, vinyl chloride), anesthetics (enflurane, halothane) and some drugs such as phenylbutazone and acetaminophene are some of the substrates of this enzyme.[16] CYP2E1 enzymes are inducible by chronic alcoholism and can render the patient susceptible to toxic effect of the compounds mentioned above. There are some evidences that showed that ethanol toxicity is caused by the production of reactive oxygen intermediate in the endoplasmic reticulum. CYP2E1 induction is very much related to the endoplasmic reticulum proliferation followed by the increase of NADPH oxidation, which resulted in the production of H_2O_2 and superoxide radicals. Increase in the lipid peroxidation lead by CYP2E1 induction is presumed to be one of the mechanism of the liver damage. CYP2E1, O_2 and NADPH will initiate a series of reactions with PUFA (polyunsaturated fatty acid) produces ROS that is thought to cause cell toxicity. Alcohol and high fat diet can cause cell toxicity by inducing CYP2E1 direct/indirectly by overproducing ROS. ROS stimulates toxicity and cell death by activating EGFR/c-Raf in the MAPK ERK pathway.[16]

CYP2E1 inhibition is one of the strategies investigated recently as an alternative approach to treat ASH (alcoholic steatohepatitis) and NASH (nonalcoholic steatohepatitis). In addition to lycopene, other natural products such as diallyl sulfide derived from garlic, phenylethylisothiocyanate and sulphoraphan (derivate of glucocynolate and crucifera), bergamottin (psoralen

found from essential oil from grapefruit and certain oranges) and dihydrocapcaisin from red chilli.[25]

In the area of drug discovery, CYP2E1 inhibitor that is currently investigated *in vivo* is chlormethiazole. In Europe chlormethiazole is already used as a therapy of alcohol withdrawal.[25]

The present study showed that lycopene has the ability to inhibit CYP2E1 in the rat microsomal model. This is an important initial information to proceed with other research to investigate the therapeutic effect of lycopene in the treatment of some conditions related to the induction of CYP2E1 such as ASH, NASH, protection the carcinogens (nitrosamines) and hepatotoxicants (paracetamol, halothane, ethanol). This study also showed that treatment with lycopene does not induce or inhibit total cytochrome P450 and CYP1A2. The result confirms the benefit of lycopene as anticarcinogens.

In conclusion, the effect of lycopene to inhibit the CYP2E1 was thought to be one of the mechanism of action of lycopene in the protection against carcinogens/ hepatotoxicants induced tissue damage that needs to be activated by CYP2E1.

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