DOSE RESPONSE AND PROTECTION EFFECT OF LYCOPENE TO REACTIVE OXYGEN SPECIES ON HUMAN CELLS

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Abstrak. *Lycopene adalah jenis karotenoid yang memiliki 40 atom karbon sebagai rantai hidrokarbon terbuka yang mengandung ikatan ganda 11 terkonjugasi dan 2 non terkonjugasi dalam struktur linear. Sumber utama lycopene adalah buah-buah warna merah dan sayuran. Lycopene dilaporkan berkontribusi mencegah kerusakan oksidatif dan mengurangi resiko kanker dan penyakit jantung koroner. Jenis penelitian ini adalah in vitro experimental. Dua jensi uji yang digunakan yaitu uji trypan blue exclusion dan MTT. Penelitian ini menggunakan sel limphoblastoid manusia (WIL2NS) sedangkan lycopene dari Sigma, USA. Pada konsentrasi rendah (0 - 10 μM) selama paparan 1 jam, lycopene tidak beracun terhadap WIL-2NS. Hal ini didukung oleh dosis aman pada kisaran 0 – 4 μM selama 2 jam paparan. Penambahan berbagai konsentrasi lycopene (2 dan 4 μM) selama 2 jam paparan dapat menurunkan sel hidup dari sel WIL-2NS pada konsentrasi berbeda dari t-BHP (0, 1 dan 7.5 μg/ml) kecuali konsentrasi lycopene 2 μM pada konsentrasi 1 μg/ml dari t-BHP dengan 98.5% + 5.5 cell hidup.*

Kata kunci: *Lycopene, Spesies Oxygen Reaktif, Efek Proteksi*

Lycopene which has molecular formula $C_{40}H_{56}$ is a lipophilic compound and is insoluble in water. Lycopene is a carotenoid which has 40 carbon atoms as open chain hydrocarbon containing 11 conjugated and 2 non-conjugated double bonds in a linear structure[7].

Figure 1
The chemical structure of lycopene [7]

The most common sources of lycopene are red fruits and vegetables. In addition to tomatoes, other foods rich in lycopene are watermelon, pink grapefruit, apricot, pink guava and papaya, as illustrated in Table 1. Cooking or food processing does not influence the loss of lycopene content significantly, and even appears to increase the content of lycopene based on total weight, as described in Table 2.

Lycopene has been reported to contribute in protecting against oxidative damage and reducing the risk of cancer and coronary heart disease[7].

Table 1
Locopene contents of common fruits/vegetables products[7]

Fruits/Vegetables	Lycopene (mg/g wet weight)
Tomatoes	8.8 – 42.0
Water melon	23.0 -72.0
Pink guava	54.0
Pink grapefruit	33.6
Papaya	20.0 – 53.0
Apricot	< 0.1

In addition to protecting against oxidative damage, lycopene may play an important role against carcinogenesis. The mechanisms of protecting against cancer attacks are by, firstly, functioning as a natural antioxidant, secondly, increasing cellular gap junction communications, thirdly, stimulating phase II enzymes involved in the activation of the antioxidant response element transcription systems, fourthly, blocking out insulin-like growth

1 *Dosen Jurusan Gizi Poltekkes Denpasar*
2 *Dosen Jurusan Keperawatan Poltekkes Denpasar*

factor-1(IGF1)-stimulated cell proliferation by generating IGF binding protein[10].

Table 2
Locopene contents of common tomato products[7]

Tomato products	Lycopene (mg/g weight)
Fresh tomatoes	8.8 – 42.0
Cooked tomatoes	37.0
Tomato sauce	62.0
Tomato paste	54.0 – 1500.0
Tomato soup	79.9
Tomato powder	1126.3 – 1264.9
Tomato juice	50.0 – 116.0
Pizza sauce	127.1
Ketchup	99.0 – 134.4

Epidemiological evidence suggests that lycopene may provide protection against cancer and other degenerative diseases such as cardiovascular disease. It was reported by **Giovannucci *et al.*** [4] that high intake of tomato products can reduce prostate cancer risk, which was confirmed in US Health Professional Follow-up Study from 1986 to 1992. Moreover, **Giovannucci *et al.***[4] revealed there was almost 35% risk reduction of prostate cancer with more than 10 servings of tomato products per week and it was found that the protective effects was stronger when the analysis was focused on more advanced and aggressive prostate cancer.

In vitro studies have indicated that lycopene is an effective antioxidant and radical scavenger[7]. **Lin *et al.***[5] reported that lycopene has ability to inactivate hydrogen peroxide and nitrogen dioxide. Furthermore, **Giovannucci *et al.***[4] revealed the ability of lycopene to significantly decrease the level of TBARS (*thiobarbituric acid reactive substances*) and DNA damage.

A fully randomized and cross over study conducted by **Rao and Shen,**[7] of twelve healthy human subjects revealed a significant increase in serum lycopene level for both ketchup and capsules intake. Subsequently, based on the results of the study, an intake of 5 to 10 mg lycopene per day is recommended.

Methods

The type of this research was *in vitro* experimental. There were two assays used in this research including the trypan blue exclusion assay which was used to monitor the growth of the cell population and the MTT assay which was used to measure the acute cell survival (cytotoxicity). This research employed a human limphoblastoid cell line (WIL2-NS) while lycopene was from Sigma, USA.

Result and discussion

Lycopene Dose Response

Figure 2 indicates the survival of WIL2-NS cells after exposure to various doses of lycopene for 1 hour. Increasing dose of lycopene decreased the cell survival of WIL2-NS cells. The dramatic decrease of the cell survival occurred at the concentration more than 10 μM of lycopene. Meanwhile at below lycopene concentration of 10 μM the cell survival still was maintained around 90 – 100%. At the concentration of 20 μM lycopene, most of the cells were dead, just only approximately 5% of the cell survived. Moreover, at the concentration of 40 μM lycopene or more there were no cells found alive. This condition was likely influenced by the presence of solvent used (benzene). It was revealed that the solvent at the same volume used as the highest dose of lycopene (160 μM) killed the cells.

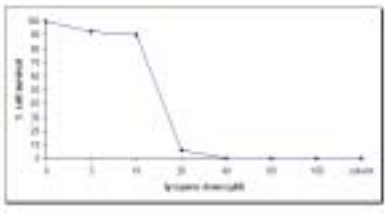

Figure 2
Cell survival following lycopene treatment for 1 hour to WIL2-NS. Cell survival was measured using the MTT assay as outlined in the Materials and Methods. Data was from single observation.

Lycopene is recognized as an effective antioxidant and radical scavenger[7]. At low concentrations (0 - 10 μM) for a 1 hour exposure, lycopene was non-toxic to WIL-2NS (Figure 3.4). This was supported by safe doses of lycopene in the range of 0 – 4 μM for a 2 hour exposure (Figure 2). Meanwhile, at higher concentrations (more than 10 μM), a dramatic decrease of the WIL-2NS cell viability occurred. This is probably due to the increasing concentration of solvent used (benzene), which was used to carry lycopene to enter the cells, and the increasing of lycopene concentration.

This research revealed that the presence of benzene in low concentration of lycopene did not affect the toxicity of cells. However, benzene at concentration of 0.13% (v/v) or more was toxic to WIL-2NS. According to **Rana and Verma**[6] benzene is a carcinogenic agent which is metabolized mainly into benzene oxide and epoxide that initiate DNA strand breaks, chromosomal damage, sister chromatid exchange and damage to mitotic spindle.

In addition to the solvent interference, lycopene may have become prooxidant at high concentrations. **Lin *et al.***[5] revealed that lycopene protect HT29 cells against DNA damage at relatively low levels (1 - 3 μM) but may have enhanced such damage at higher concentrations (4 - 10 μM) using the comet assay. **Yeh and Hu**[12] also reported that lycopene behaved as prooxidant in Hs68 cells treated with 2,2'-*azobis*[2,4-*dimethyl valeronitrile* (AMVN) at a concentration of 20 μM.

Figure 3 illustrates various doses of lycopene (0 – 8 μM) used to treat WIL2-NS cells for 2 hours against its cell survival. The figure indicates that the safe doses of lycopene for 2 hours exposure were achieved at 4 μM (90.17% + 2.99) and lower. The dose of 6 and 8 μM of lycopene were considered unsafe for WIL2-NS cells, since those resulted in the cell survival lower than 90 %.

Dilution with the growth medium then was conducted to deliver lycopene into the cells.

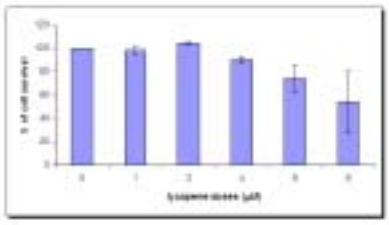

Figure 3
Cell survival following lycopene treatment for 2 hours to WIL2-NS. Cell survival was measured using the MTT assay as outlined in the Materials and Methods. Bars represent means- + Standard Error from 3 independent experiments

Protection Effect of Lycopene

Figure 4 shows the protection effect of lycopene against *t*-BHP as ROS generator to WIL2-NS cells. The addition of various concentration of lycopene (2 and 4 μM) for 2 hours exposure was likely to decrease the cell survival of WIL2-NS cells at different concentration of *t*-BHP (0, 1 and 7.5 μg/ml respectively), except the concentration of 2 μM of lycopene at 1 μg/ml of *t*-BHP with 98.5% + 5.5 cell survival. Even though there was no statistically significant difference ($P>0.05$), a trend to a protection effect of lycopene is shown by the differences between lycopene at 4 μM and *t*-BHP at 7.5 μg/ml exposure alone and the observed combination between both of them. As can be seen in Figure 3.9 the cell killing of *t*-BHP alone (7.5 μg/ml) was 24.5% + 2.4, while the cell killing of lycopene (4 μM) for 2 hour was 17.4% + 13.3. Following this result, it was predicted that the cell killing of combination between lycopene and *t*-BHP would be approximately 42%. However, the observed cell killing by the combination of lycopene and *t*-BHP was 34.9% + 4.8 which was lower than the predicted combination cell killing.

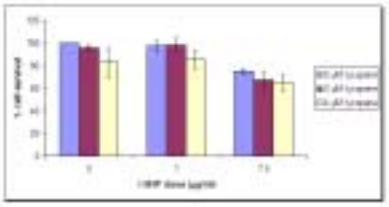

Figure 4
Protection effect of lycopene for 2 h treatment to WIL2-NS cells. Bars represent means- + standard error from 3 independent experiments

Lycopene has been shown to have higher antioxidant activity than β-carotene[10]. Because of its high number of conjugated double bonds, lycopene becomes a most potent radical scavenger[4]. The participation of lycopene in reactions with free radicals is probably intimately linked with disruption and breakdown of the primary structure of lycopene[3].

Pre-incubation of lycopene was conducted for 1 hour to allow the cells to take up and accumulate lycopene within cell membrane against oxidative insult. Due to extreme hydrophobicity properties, lycopene may react with reactive oxygen species in the hydrophobicity inner core of the membrane[3]. Moreover, co-incubation of lycopene for 1 hour, followed by t-BHP exposure, was expected to stabilize ROS induced by *t*-BHP by lycopene chain breaking.

This research found an antioxidant potential of lycopene with a 7% cell killing difference between exposure of lycopene and *t*-BHP alone and observed combination of both. That there was no significant protection of lycopene may be due to low lycopene concentration uptake of cells and the type of oxidative agent used.

This research used benzene as a vehicle to deliver lycopene into the cells. The high toxicity of benzene could have influenced the amount of lycopene transfered into the cells, eventually resulting in low lycopene accumulation within the membrane of cells and hence not protecting the cells from *t*-BHP oxidation. It was found in this research that the safe concentrations of benzene to WIL2-NS cells is 0.13% (v/v) and lower (data not shown). It means the amount of lycopene which was delivered into the cells was limited to those doses. It was not optimal to obtain a sufficient amount of lycopene which could be delivered to protect the cells. **Lin *et al*.**[5] demonstrated that the use of fetal bovine serum (FBS), as a vehicle, improved the uptake and stability of lycopene into two prostate cancer cell lines, compared to the use of *tetrahydrofuran* (THF), THF containing *butylated hydroxytoluen* (BHT), *methyl-β-cyclodextrin* (M-β-CD) and micelles.

The use of different oxidative agents may generate different protection effects of lycopene. In this research, lycopene did not significant protect (P>0.05) WIL2-NS from *t*-BHP-induced damage. It was reported by **Yeh and Hu**[11] that lycopene did not significantly protect Hs68 cells from DNA damage induced by three radical generators, 2,2'-*azobis*[2,4-*dimethylvaleronitrile*] (AMVN); 2,2'-*azobis*[2-*amidinopropane*] *dihydrochloride* (AAPH) and *ferric nitrilotriacetate* (Fe/NTA). However, lycopene protected cultured rat hepatocytes against carbon tetrachloride injury and death [10]. The probable reason was polarity discrepancy between the oxidative agents and lycopene to interact each other.

Conclusion

Lycopene has been shown to have high antioxidant activity, because of its high number of conjugated double bonds, lycopene becomes a most potent radical scavenger. The participation of lycopene in reactions with free radicals is probably intimately linked with disruption and breakdown of the primary structure of lycopene. There was no significant protection of lycopene may be due to low lycopene concentration uptake of cells and the type of oxidative agent used

Reference

1. ATCC. 2000. Catalogue of Cell Lines and Hybridomas. A.T.C. Collection, editor. www.atcc.org.
2. Bohm, F., J.H. Tinkler, and T.G. Truscott. 1995. Carotenoids protect against cell membrane damage by the nitrogen dioxide radical. Nature Medicine. 1:98-99.
3. Clinton, S.K. 1998. Lycopene: chemistry, biology, and implications for human health and disease. Nutr Rev. 56:35-51.
4. Giovannucci, E., A. Ascherio, E.B. Rimm, M.J. Stampfer, G.A. Colditz, and W.C. Willett. 1995. Intake of Carotenoids and Retino in Relation to Risk of Prostate Cancer. jnci. 87:1767-1776.
5. Lin, C.Y., C.S. Huang, and M.L. Hu. 2007. The use of fetal bovine serum as delivery vehicle to improve the uptake and stability of lycopene in cell culture studies. British Journal of Nutrition. 98:226-232.
6. Rana, S.V., and Y. Verma. 2005. Biochemical toxicity of benzene. J Environ Biol. 26:157-68.
7. Rao, A.V., and S. Agarwal. 1999. Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: A review. Nutrition Research. 19:305-323.
8. Rao, A.V., and H. Shen. 2002. Effect of low dose lycopene intake on lycopene bioavailability and oxidative stress. Nutrition Research. 22:1125-1131.
9. Tinkler, J.H., F. Bohm, W. Schalch, and T.G. Truscott. 1994. Dietary carotenoids protect human cells from damage. J Photochem Photobiol B. 26:283-5.
10. Wang, X.D. 2005. Can Smoke-Exposed Ferrets Be Utilized to Unravel the Mechanisms of Action of Lycopene? Journal of Nutrition. 135:2053-2056.
11. Yeh, S.L., and M.L. Hu. 2000. Antioxidant and pro-oxidant effects of lycopene in comparison with ß-carotene on oxidant-induced damage in Hs68 cells. The Journal of Nutritional Biochemistry. 11:548-554.