

The use of fetal bovine serum as delivery vehicle to improve the uptake and stability of lycopene in cell culture studies

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Tetrahydrofuran (THF) has commonly been used to deliver carotenoids to cells but the use of THF is associated with cytotoxicity and low uptake efficiency of carotenoids. Here, we used fetal bovine serum (FBS) as the delivery vehicle for lycopene in comparison with THF, THF containing 0.0025% butylated hydroxytoluene (THF/BHT), methyl- β -cyclodextrin (M- β -CD) and micelles in two human prostate cancer cell lines, DU145 and PC-3. Lycopene (10 μ M) solubilized in THF/BHT and then diluted in FBS at ratios of 5 and 10 gave the highest lycopene uptake in DU145 cells. Using a dilution factor of 10, we found that lycopene (10 μ M) carried in FBS in a cell-free system led to significantly less loss of lycopene than in THF, THF/BHT and M- β -CD within 24 h of incubation. Lycopene solubilized in micelles was more stable than that in FBS within 24 h, but the micelle itself led to marked cytotoxicity to DU145 cells. Lycopene at 10 μ M in FBS led to significantly higher uptake of lycopene in both cell lines than that in THF, THF/BHT or M- β -CD within 24 h of incubation. When FBS was replaced with lipoprotein-deficient serum, the uptake of lycopene by DU145 cells was markedly decreased and was not significantly different from that of THF or THF/BHT. These results demonstrate that FBS is superior to THF, THF/BHT, M- β -CD and micelles as a delivery vehicle for lycopene in prostate cell lines and that the lipoprotein of FBS is likely responsible for the improved stability and cellular uptake of lycopene.

Cellular uptake: Delivery vehicles: Fetal bovine serum: Lycopene

Carotenoids are a class of natural pigments that possess antioxidant activities both *in vitro* and *in vivo* (Stahl & Sies, 1996; Clinton, 1998; Bramley, 2000). Among the more than 600 carotenoids found in nature, β -carotene and lycopene are the two most abundant carotenoids present in the human body (Sies & Stahl, 2003). Studies have suggested that higher intakes of lycopene are associated with a reduced risk of several types of cancer, such as prostate cancer and hepatoma (Stahl & Sies, 1996; Clinton, 1998; Kotake-Nara *et al.* 2001). Cell culture studies are useful for elucidating the mechanisms of action of lycopene, and tetrahydrofuran (THF) has commonly been used to deliver lycopene to cells (Bertram *et al.* 1991; Conney *et al.* 1993; Huang *et al.* 2005; Tang *et al.* 2005; Yeh *et al.* 2005). However, the use of THF as the solvent for carotenoids including lycopene has repeatedly been questioned because of its disadvantages in cell culture studies (Gross *et al.* 1997; Wang *et al.* 2004; Rodriguez *et al.* 2005). For instance, THF oxidizes readily in culture media, which can lead to instability of lycopene and result in cytotoxicity (Xu *et al.* 1999; Williams *et al.* 2000; Shahrzad *et al.* 2002; Hurst *et al.* 2004; O'Sullivan *et al.* 2004) so that the final concentration of THF must be below 0.5% (Huang *et al.* 2005; Tang *et al.* 2005; Yeh *et al.* 2005). In addition, lycopene dissolved in THF has low cellular association and high standard deviation

(Shahrzad *et al.* 2002). Therefore, attempts have been made by using vehicles other than THF for delivering lycopene to cells including dimethyl sulphoxide (DMSO), cyclodextrin, lipoprotein, micelle and beadlets (Martin *et al.* 1996, 1997; Stivala *et al.* 1996; Garrett *et al.* 1999; Pfitzner *et al.* 2000; Lancrajan *et al.* 2001; Da Violante *et al.* 2002; Gamer *et al.* 2002; Vertzoni *et al.* 2006). Each of these vehicles provides certain advantages but also suffers limitations such as cytotoxicity, poor solubility and crystallization in the medium (Stivala *et al.* 1996; Xu *et al.* 1999; Lancrajan *et al.* 2001; Shahrzad *et al.* 2002; O'Sullivan *et al.* 2004; Palozza *et al.* 2006). For example, DMSO has been used for delivering carotenoids into cells because it readily penetrates cell membranes and is relatively non-cytotoxic (Da Violante *et al.* 2002; Rodriguez *et al.* 2005). However, DMSO is also unstable and has low solubility for carotenoids (Gross *et al.* 1997). Indeed, the half-life of lycopene in aqueous/organic solvents is only 2 h, which is inappropriate for the studies of carotenoids (Xu *et al.* 1999). The use of Tween 40 and Tween 80 to deliver phytochemicals including lycopene to cultured cells has been advocated (O'Sullivan *et al.* 2004), but the method is somewhat laborious and may require extra care to avoid oxidation of carotenoids, as it requires drying the solvent twice (under nitrogen) followed by filtration.

Abbreviations: BHT, butylated hydroxytoluene; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; M- β -CD, methyl- β -cyclodextrin; THF, tetrahydrofuran.

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Several other vehicles for delivering carotenoids have been reported. For instance, cyclodextrin-complexed carotenoids have been shown to protect carotenoid from reactive oxygen species, but such complexes result in considerable decrease in antioxidant ability of the carotenoid (Polyakov *et al.* 2004). The water-dispersible carotenoid beadlets have been shown to be non-toxic and relatively stable to cultured cells (Shahzad *et al.* 2002), but these beadlets are not commercially available. Another useful vehicle for delivering carotenoids into cells *in vitro* is micelles because, as reported by Xu *et al.* (1999), they are non-cytotoxic and can stabilize carotenoids such as lycopene. However, Martin *et al.* (1997) have found that their micelle preparation does not stabilize carotenoids, and they have advocated the use of human lipoproteins for solubilizing and stabilizing carotenoids. Indeed, carotenoids including lycopene have been suggested to be transported by lipoprotein in man (Cardinault *et al.* 2005; Palozza *et al.* 2006). It is interesting to note that Williams *et al.* (2000) have used bovine serum from steer supplemented with β -carotene as a delivery vehicle in cell culture and have obtained satisfactory results, as compared to other vehicles.

In the present report, we made use of the fact that fetal bovine serum (FBS) contains lipoproteins, which may be a convenient and useful vehicle for delivering lycopene into cells in culture. To test this possibility, we solubilized lycopene in THF containing butylated hydroxytoluene (BHT), diluted the THF/BHT-lycopene mixture in FBS at different ratios, and selected an appropriate ratio for studying the stability and cellular uptake of lycopene in two prostate cancer cell lines. Although Martin *et al.* (2000) have used ethanol-THF to dissolve carotenoids and mixed them with FBS to deliver carotenoids into human aortic endothelial cells, they have not described the details, nor have they compared the method with other delivery vehicles for carotenoids. Here, we compared the FBS dilution method of lycopene with other solubilization vehicles for lycopene including THF, THF containing 0.0025% BHT (THF/BHT), methyl- β -cyclodextrin (M- β -CD) and micelles in cultured cells, and we measured the cytotoxicity of these delivery vehicles and the stability and cellular uptake of lycopene in these vehicles. We used two human prostate cancer cell lines, DU145 and PC-3, both of which are androgen-independent, because lycopene is most frequently reported to prevent prostate cancer (Kristal & Cohen, 2000).

Material and methods

Materials

The human androgen-independent prostate cancer cell lines DU145 and PC-3 cells were purchased from the Food Industry Research and Development Institute, Taiwan. All chemicals used are of the highest grade. THF, THF containing 0.0025% BHT, and FBS were obtained from Merck (Darmstadt, Germany). Dulbecco's modified Eagle's medium (DMEM), non-essential amino acid, penicillin/streptomycin, sodium pyruvate and trypsin were from Gibco/BRL (Grand Island, NY, USA). Lycopene and lipoprotein-deficient serum were from Sigma (St Louis, MO, USA) and M- β -CD was from Fluka (Steinheim, Switzerland).

Preparation of lycopene in various vehicles

Stock solutions of lycopene (10 mM) in THF or THF/BHT were freshly prepared before each experiment and were added to cell culture medium at a final concentration of 10 μ M which was determined to be between 9.0 and 10 μ M using HPLC as described later. To prepare the FBS-carried lycopene, we adopted the method of Martin *et al.* (2000) for preparing FBS-incorporated carotenoids with some modification. Lycopene was first solubilized in THF/BHT to form a stock solution of 10 mM-lycopene, which was then diluted with FBS at indicated ratios (1:4, 1:9, 1:19, 1:49). After vortexing for 1 min, this THF/FBS-lycopene preparation was added to cell culture to achieve a final concentration of 10 μ M-lycopene in the culture medium. The final concentrations of THF and BHT in the culture medium were 0.1% and 0.000025%, respectively.

For solubilization of lycopene in M- β -CD, a quantity of 5 mg lycopene was mixed with 1 ml absolute ethanol (5 mg/ml), to which was added 0.5 g M- β -CD/ml solution, and the organic solvent was removed under a stream of nitrogen in the dark. The residue (1 g) was re-dissolved in 2 ml deionized water to achieve a final concentration of 9.3 mM-lycopene and stored at -80°C .

The method for preparing micelle-solubilized lycopene (1 mM) was based on that reported by Xu *et al.* (1999). First, 13 μ l L- α -phosphatidylcholine (16.7 μ M), 100 μ l oleic acid (33.3 μ M), 100 μ l monoolein (100 μ M) and 100 μ l 1 mM-lycopene (dissolved in THF/BHT) were combined, and the organic solvent was removed under nitrogen in the dark. Next, 10.76 mg sodium taurocholate (2 mM) was added to 10 ml of cell culture medium (DMEM) containing 10% FBS, and this mixture was used to dissolve the lycopene residue. The mixture was sonicated in a waterbath at room temperature for 15 min to form micelles containing lycopene and sterilized by passage through a 0.22 μ m filter.

Cell culture and uptake of lycopene

DU145 cells and PC-3 cells were grown in a 10 cm dish with 7 ml DMEM containing 10% (v/v) FBS, 1% non-essential amino acid, 1% sodium pyruvate, 1% penicillin (10 000 unit/ml)-streptomycin (10 mg/ml) and 0.37% NaHCO₃ (w/v). The cells were incubated in a humidified atmosphere of 5% CO₂ and 95% air at 37°C and harvested at approximately 90% confluence (10⁶ cells/dish), after which the culture medium was decanted and replaced with fresh medium (5 ml). Lycopene dissolved in THF, THF/BHT, M- β -CD or FBS was added to cells at a final concentration of 10 μ M (three Petri dishes/group). The cells (approximately 10⁶ cells/dish) were incubated for 2, 6, 12 and 24 h at 37°C in the dark. Cellular lycopene was extracted and analysed by HPLC, as described later.

Stability of lycopene in cell-free medium

The stability of the different lycopene solutions was measured under cell culture conditions in DMEM medium containing 10% (v/v) FBS, 1% non-essential amino acid, 1% sodium pyruvate, 1% penicillin (10 000 unit/ml)-streptomycin (10 mg/ml). The medium containing 10 μ M lycopene from different delivery vehicles was incubated at 37°C for 0, 2, 4,

6, 12 and 24 h in the dark, and the remaining lycopene was extracted and analysed by HPLC, as described later.

Determination of lycopene in culture medium and in cells

The cell-free culture medium (1 ml) was added to 0.4 ml mixture of hexane and absolute ethanol (2:1, v/v) and vortexed for 1 min. The mixture was centrifuged at 1200 g for 4 min, and the hexane layer, which contains lycopene, was transferred to an Eppendorf tube and stored at -80°C . The extracted lycopene was quantified by HPLC (Merck-Hitachi system, Darmstadt, Germany). The chromatography conditions were as follows: a C18 column, 250×4.6 mm, $5 \mu\text{m}$ was eluted at 1.5 ml/min with isocratic mobile phase (methanol–toluene, 75:25, v/v). The injection volume was 20 μl , and lycopene was monitored at 470 nm (Rundhaug *et al.* 1988).

For measurement of cellular lycopene, the culture medium was decanted and the cells were washed with PBS three times. The attached cells were scraped and resuspended with 1 ml PBS. The cells were transferred from the dishes to centrifuge tubes and a 2 ml mixture of hexane and absolute ethanol (2:1, v/v) was added (Rundhaug *et al.* 1988). After sonication in a water bath at room temperature for 1 min, the mixture was centrifuged (1200 g, 4 min). The hexane layer was transferred to an Eppendorf tube and stored at -80°C until assay for lycopene using HPLC, as described earlier.

Determination of cellular uptake of lycopene

The cellular uptake of lycopene is defined as the difference of total amounts of lycopene absorbed by the cells (determined at 37°C) minus the amounts of lycopene adherent to the cell surface (determined at 4°C) (Huang *et al.* 2007) for up to 24 h. The amounts of lycopene adherent to the cell surface were found to be approximately 6% of the total lycopene. The percentage uptake efficiency of lycopene was calculated by the formula: $[(\text{nmol lycopene}/10^6 \text{ cells}) / \text{or} / (\text{nmol lycopene}/\text{dish}) \times 100]$ (Williams *et al.* 2000), in which the numerator is the cellular uptake of lycopene, and the denominator is the amount of lycopene added to the medium (10 μM or 50 nmol lycopene in 5 ml medium per 10 ml Petri dish).

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenol tetrazolium bromide assay

The cell viability of lycopene delivery vehicles was determined by microtitration assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenol tetrazolium bromide. DU145 cells were seeded at a density of 5×10^4 cells per well in a twenty-four-well multititre plate in DMEM for 24 h, and the culture medium was replaced with a delivery vehicle (THF, THF/BHT, FBS or M- β -CD) at 0.1–1.0% THF or M- β -CD and then incubated for 24 h. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenol tetrazolium bromide (0.25 mg/ml) was added to each well and incubated for 1 h, after which the liquid was removed, and dimethyl sulphoxide was added to dissolve the solid residue. The optical density at 570 and 492 nm of each well was then determined using a microplate reader (FLUOstar OPTIMA; BMG Labtechnologies, Germany).

Statistical analysis

Values are expressed as means and standard deviations and analysed by one-way ANOVA followed by Duncan's multiple range test for comparisons of group means. $P < 0.05$ was considered significant.

Results and discussion

In the present study, we made use of the fact that FBS contains lipoproteins that are necessary for the transport of carotenoids (Cardinault *et al.* 2005; Palozza *et al.* 2006). Although FBS has been used to deliver carotenoids including lycopene to cultured cells (Martin *et al.* 2000), no experimental data on the stability and cellular uptake have been presented, and no comparisons of the FBS method with other delivery methods have been made. We first tested the appropriate dilution ratio of lycopene to FBS for delivering lycopene to DU145 cells. The stock solution of lycopene (10 mM in THF/BHT) was diluted with FBS at 5-, 10-, 20- or 50-fold. After incubation for 12 h, we found that a dilution factor of 5 or 10 gave higher lycopene uptake than the dilution factor of 20 or 50 (Fig. 1). Because the uptake of lycopene by 10-fold dilution was slightly higher than that by 5-fold dilution, we adopted the 10-fold dilution for the studies of stability and cellular uptake of lycopene in comparison with other delivery vehicles for lycopene. For convenience, this THF/FBS delivery system is referred to as FBS.

Stability of lycopene in different delivery vehicles in cell-free systems

Fig. 2 shows the stability of lycopene in various vehicles in the cell-free system during incubation for 24 h at 37°C in the dark. Degradation of lycopene in different delivery vehicles increased with the incubation time, and the degradation rates of lycopene at 24 h were in the order: THF > THF/BHT > M- β -CD > FBS > micelles. Approximately 80 and 50% lycopene remained for micelles and FBS, respectively, as the delivery

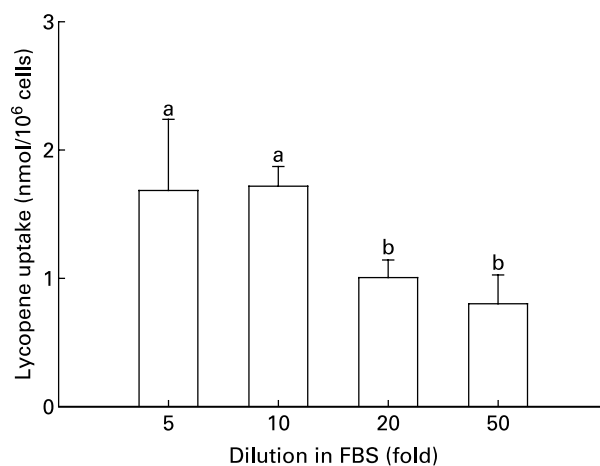


Fig. 1. Lycopene uptake by DU145 human prostate cancer cells incubated at 37°C for 12 h with lycopene (dissolved in tetrahydrofuran) diluted with fetal bovine serum (FBS) as delivery vehicle. Values are means with their standard deviations depicted by vertical bars ($n \geq 3$). ^{a,b} Mean values with unlike letters were significantly different ($P < 0.05$).

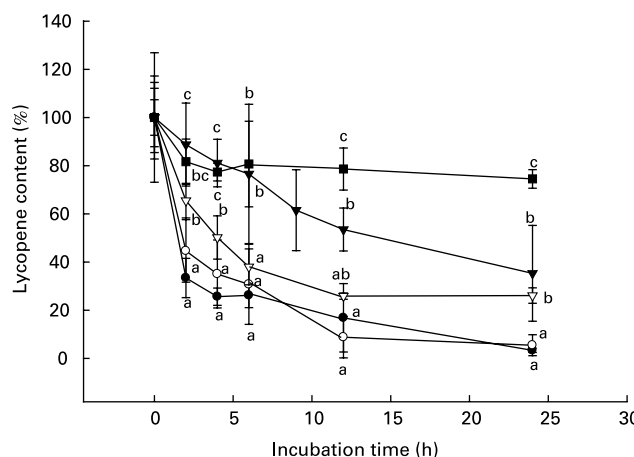


Fig. 2. Effects of tetrahydrofuran (●), tetrahydrofuran/butylated hydroxytoluene (○), fetal bovine serum (▼), methyl- β -cyclodextrin (▽) and micelles (■) on the stability of lycopene in cell-free systems. Incubations were carried out in the dark at 37°C for 2, 4, 6, 12 and 24 h. Values are means with their standard deviations depicted by vertical bars. ^{a,b,c} Mean values at the same time-point with unlike letters were significantly different ($P < 0.05$).

vehicle. This experiment shows that micelles can better stabilize lycopene than can other delivery vehicles used in the present study. It is unclear why Martin *et al.* (1997) reported that their micelle preparation does not stabilize carotenoids, since their method for preparing micelles is essentially the same as that used by us (Xu *et al.* 1999).

Cytotoxicity of lycopene solubilized in various delivery vehicles

Before we determined the uptake efficiency of lycopene, we examined the cytotoxicity of THF, THF/BHT, FBS,

M- β -CD and micelles to prostate cancer cells in the absence of lycopene. In Fig. 3, the concentrations (0.1 and 1.0 %) of THF, THF/BHT and FBS referred to their solvent (THF). M- β -CD and micelles referred to the vehicle itself, as both M- β -CD and micelles were dried and re-suspended in deionized water (M- β -CD) or the culture medium DMEM (micelles) before adding to the cells. We found that FBS (containing 0.1 or 1.0 % THF) and M- β -CD were not cytotoxic to the cells incubated for 24 h, whereas THF and THF/BHT at 1.0 % (but not 0.1 %) caused a significant decrease in cell viability. Unexpectedly, the micellar preparation caused strong cytotoxicity to DU145 cells, and this effect was already evident at 2 h of incubation. We also observed cytotoxicity in PC-3 cells induced by micelles, but we only focused on DU145 because the uptake of lycopene in FBS was somewhat higher in DU145 cells than in PC-3 cells.

It is unclear why the micellar preparation (in DMEM) was cytotoxic, but it might be related to the fatty acids (e.g. phosphatidylcholine) contained in the micelles, which could undergo oxidation in cell culture medium. It is noteworthy that cell culture studies are normally carried out under abnormal conditions known as 'culture shock', where cells are exposed to high oxygen tension and to free metal ions in the medium (Halliwell, 2003). As a result of the cytotoxicity, we did not further study the uptake of lycopene by micelles.

Cellular uptake of lycopene in different delivery vehicles

Lycopene delivered by FBS achieved a higher cellular uptake than lycopene by other delivery vehicles during the 24 h incubation in both DU145 cells (Table 1) and PC-3 cells (Table 2). At 12 h of incubation, lycopene uptake in DU145 cells

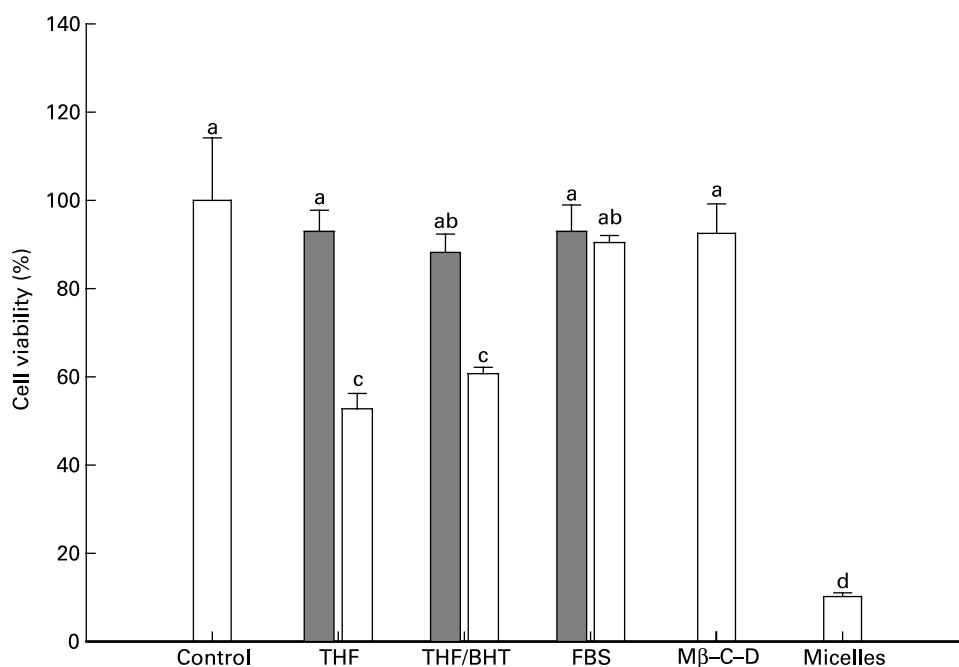


Fig. 3. Cell viability of DU145 cells incubated at 37°C for 24 h with tetrahydrofuran (THF), THF/butylated hydroxytoluene (THF/BHT), fetal bovine serum (FBS), methyl- β -cyclodextrin (M- β -CD) and micelles. For THF, THF/BHT and FBS, two levels were used (□, 0.1%; ■, 1.0%), which referred to their solvent concentration (THF). M- β -CD and the micellar preparation referred to the vehicle itself, since both vehicles were dried and re-suspended in deionized water and the culture medium (Dulbecco's modified Eagle's medium), respectively, before adding to the cells. Values are means with their standard deviations depicted by vertical bars. ^{a,b,c,d} Mean values with unlike letters were significantly different ($P < 0.05$).

Table 1. Uptake of lycopene (nmol/10⁶ cells) delivered to DU145 cells by tetrahydrofuran (THF), THF/butylated hydroxytoluene (BHT), fetal bovine serum (FBS) and methyl- β -cyclodextrin (M- β -CD) for 2, 6, 12 and 24 h

(Mean values and standard deviations)

Delivery methods	Incubation time (h)							
	2		6		12		24	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
THF	0.10 ^b	0.02	0.15 ^a	0.06	0.90 ^b	0.43	0.09 ^b	0.04
THF/BHT	0.14 ^{ab}	0.03	0.18 ^a	0.03	1.12 ^b	0.32	0.12 ^b	0.04
FBS	0.18 ^a	0.04	0.27 ^a	0.13	1.72 ^a	0.29	0.37 ^a	0.15
M- β -CD	–	–	–	–	1.18 ^b	0.01	–	–

^{a,b} Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).**Table 2.** Uptake of lycopene (nmol/10⁶ cells) delivered to PC-3 cells by tetrahydrofuran (THF), THF/butylated hydroxytoluene (BHT), fetal bovine serum (FBS) and methyl- β -cyclodextrin (M- β -CD) for 2, 6, 12 and 24 h

(Mean values and standard deviations)

Delivery methods	Incubation time (h)							
	2		6		12		24	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
THF	0.13 ^b	0.01	0.22 ^b	0.02	0.10 ^c	0.03	0.05 ^b	0.01
THF/BHT	0.18 ^a	0.01	0.19 ^{ab}	0.02	0.07 ^c	0.01	0.18 ^a	0.04
FBS	0.16 ^a	0.02	0.23 ^a	0.01	0.38 ^a	0.03	0.22 ^a	0.08
M- β -CD	–	–	–	–	0.22 ^b	0.05	–	–

^{a,b,c} Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).

delivered by FBS was significantly higher ($P < 0.05$) than that delivered by THF, THF/BHT and M- β -CD (by 91, 54 and 46 %, respectively) (Table 1), while the uptake in PC-3 cells delivered by FBS was significantly higher ($P < 0.05$) than that delivered by THF, THF/BHT and M- β -CD (by 280, 443 and 73 %, respectively) (Table 2). Regardless of the delivery vehicle, the lycopene uptake by DU145 cells at 12 h of incubation was higher than that by PC-3 cells, but at 24 h of incubation such differences had disappeared.

Effects of lipoprotein on lycopene uptake in fetal bovine serum

Because lycopene has been suggested to be transported by lipoprotein in man (Cardinault *et al.* 2005; Palozza *et al.* 2006), we assumed that the lipoprotein fraction of FBS is responsible for the increased uptake efficiency of lycopene in FBS. To test this assumption we employed lipoprotein-deficient serum as the delivery vehicle for lycopene and incubated with DU145 cells in comparison with FBS, THF and THF/BHT as vehicle. Lycopene solubilized in THF was as mixable in the lipoprotein-deficient serum as it was in FBS. This lipoprotein-deficient serum–lycopene mixture was added to culture medium immediately, and we observed no apparent lycopene loss or detectable cytotoxicity in using this mixture (data not shown). After incubation for 12 h, the uptake efficiency of lycopene delivered by lipoprotein-deficient serum (1.8 (SD 0.2) %) was indeed significantly lower than that by FBS (3.4 (SD 0.6) %, $P < 0.05$) and was

not significantly different from that by THF or THF/BHT (1.8 (SD 0.9) and 2.2 (SD 0.6) %, respectively) (Table 3). The present results strongly indicate that the lipoprotein fraction of FBS plays an important role in delivering lycopene to cells. The present results in lycopene are in accord with those in other carotenoids or in α -tocopherol. For instance, Martin *et al.* (1997) have

Table 3. Effect of lipoprotein-deficient serum (DL-S) in comparison with fetal bovine serum (FBS), tetrahydrofuran (THF) and THF/butylated hydroxytoluene (BHT), as delivery vehicle on lycopene uptake in DU145 cells*

(Mean values and standard deviations)

Delivery methods	Lycopene uptake (nmol/10 ⁶ cells)†		Uptake efficiency (%)‡	
	Mean	SD	Mean	SD
THF	0.90 ^b	0.43	1.8 ^b	0.9
THF/BHT	1.12 ^b	0.32	2.2 ^b	0.6
FBS	1.72 ^a	0.29	3.4 ^a	0.6
DL-S	0.92 ^b	0.05	1.8 ^b	0.2

^{a,b} Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).* Cells were incubated in a 10 cm Petri dish containing 5 ml of a delivery vehicle for 12 h ($n \geq 3$).

† The cellular uptake of lycopene is defined as the difference of total amounts of lycopene absorbed by the cells (determined at 37°C) minus the amounts of lycopene adherent to the cell surface (determined at 4°C).

‡ The percentage uptake efficiency of lycopene was calculated by the formula: [(nmol lycopene/10⁶ cells)/(nmol lycopene/dish) \times 100] (see Methods).

reported that lipoproteins represent a stable vehicle for delivery of β -carotene and α -tocopherol to HepG2 human liver cells. Interestingly, Williams *et al.* (2000) have shown that β -carotene-enriched serum obtained from an Angus steer fed 200 mg β -carotene for 1 week provides a stable and physiological approach to carotenoid treatment of cells in culture.

Conclusions

The present study has demonstrated that the use of FBS for delivering lycopene into the two prostate cancer cell lines is superior to the use of THF, THF/BHT, M- β -CD and micelles. The present results further indicate that the lipoprotein of FBS is likely responsible for the improved stability and cellular uptake of lycopene. Although lycopene delivered in FBS is only relatively stable (65 and 50% remaining at 9 and 12 h of incubation), this delivery vehicle represents a simple and useful system for investigating the physiological and toxicological properties of lycopene. It remains to be investigated as to whether this FBS delivery method for lycopene is suitable for *in vitro* studies in cell lines other than the prostate cancer cells.

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