

PROMOTIVE EFFECTS OF HYPERTHERMIA ON THE INHIBITION OF DNA SYNTHESIS IN EHRlich ASCITES TUMOR CELLS BY EICOSAPENTAENOIC AND DOCOSAHEXAENOIC ACIDS

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Aim: To evaluate inhibitory effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on DNA synthesis in combination with hyperthermia *in vitro*. **Methods:** A suspension of Ehrlich ascites tumor cells (EAT) was mixed with DHA or EPA in a glass tube, heated at 37 °C, 40 °C, or 42 °C for 1 h in a water bath, and cultured at 37 °C for 19 or 96 h. DNA synthesis was assayed by monitoring of the incorporation of [³H]-thymidine into the acid-insoluble fraction. DHA or EPA incorporated into EAT cells was extracted and measured by thin-layer chromatography and gas-liquid chromatography. **Results:** The inhibition of DNA synthesis by EPA or DHA increased markedly upon the treatment at 42 °C and 40 °C compared to that at 37 °C. At 37 °C, inhibitory action of EPA was more potent than that of DHA at low concentrations (at 50 μM — DNA synthesis level: EPA, 63.1%; DHA, 87.9%), whereas inhibitory action of DHA was higher at 150 μM (16.7%, 4.4%, *ibid.*). The effect of DHA compared to EPA was more marked at 40 °C (29.0%, 19.2% at 100 μM) or 42 °C (19.7%, 10.6% at 100 μM). Evaluation of DNA synthesis rate in the cells treated for 1 h by EPA or DHA with the next culturing of EAT cells for 19 h resulted in the enhanced inhibitory activity of EPA even at concentrations as low as 50 μM at either 37 °C (0.5%, 11.3%) or 42 °C (0.6%, 4.5%), which in these conditions was higher than that of DHA. At the same time the rate of incorporation of EPA in EAT cells at 37 °C or 42 °C was lower than that of DHA. **Conclusion:** Administration of DHA or EPA *in vitro* significantly inhibit DNA synthesis, and such effect is enhanced by combination of PUFAs with hyperthermia.

Key Words: eicosapentaenoic acid, docosahexaenoic acid, hyperthermia, cytotoxic activity, DNA synthesis.

Polyunsaturated fatty acids (PUFAs) are widely known to possess antitumor activity [1–5] since Nakahara reported their suppressive effect against the growth of tumor cells implanted in mice [6]. The PUFAs inhibit either the DNA synthesis [2] or proliferation of tumor cells [1, 3, 4]. Hyperthermia is receiving attention as effective mean in combined cancer treatment. Exposure of cultured tumor cells to temperatures above 41 °C results in inhibition of the DNA synthesis [7, 8] and proliferation of tumor cells [9, 10]. Combined effect of PUFAs and hyperthermia has been first demonstrated by Kageyama et al. [11]. Oleic acid and linoleic acid (at the concentration of 300 μM) decrease DNA synthesis in Ehrlich ascites tumor (EAT) cells by 42.2% and 25.2% respectively upon 19 h culturing at 37 °C, and by 17.2 and 8.4% at 42 °C compared to EAT cells treated in the absence of fatty acid. The cytotoxic activities of fatty acids are shown to augment according to the increase in the number of double bonds especially in PUFAs consisting of more than 18 carbon atoms [2, 12]. Therefore, inhibitory activity of eicosapentaenoic acid (EPA, 20 : 5, n-3) and docosahexaenoic acid (DHA, 22 : 6, n-3), possessing 5 and 6 double bonds, respectively, on either the DNA

synthesis or tumor cell growth are stronger than those of other fatty acids such as gamma-linoleic acid (18 : 2, n-6) and arachidonic acid (20 : 4, n-6).

In the present study, in order to exceed beyond the combined effect of oleic acid or linoleic acid and hyperthermia [11] we used EPA and DHA. A combined effect of EPA or DHA and hyperthermia on tumor cells seems not to be demonstrated yet. We studied whether an inhibitory effect of EPA or DHA on the DNA synthesis in tumor cells is markedly enhanced by a combination with hyperthermia. Furthermore, the relationship between the inhibitory activity of the fatty acids and their intracellular accumulation was investigated.

MATERIALS AND METHODS

Cell culture. Ehrlich ascites tumor cells were obtained from female ICR mice with tumor transplants. After washing, cells were suspended in Eagle's minimum essential medium (MEM, Nakarai Tesque, Kyoto, Japan), supplemented with 10% fetal bovine serum (FBS, Gibco Laboratory, USA) and maintained by culturing in a humidified atmosphere of 5% CO₂ at 37 °C.

Hyperthermic treatment. The cells were suspended in MEM with 10% FBS. EPA and DHA (Sigma Chemical Co. St. MO) were separately sonicated and dispersed in the culture medium at various concentrations. The cell suspension and a suspension containing the test substance were mixed in a glass tube (14 mm i.d. x 40 mm); the total volume was 1 ml, which contained 10 or 1 x 10⁴ cells for 20- or 96 h culturing after the heat treatment, respectively. Cells in a tightly

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Abbreviations used: DHA — docosahexaenoic acid; DPO — diphenylloxazole; EAT — Ehrlich ascites tumor; EPA — eicosapentaenoic acid; FBS — fetal bovine serum; MEM — minimum essential medium; POPOP — 1,4-bis-2-(4-methyl-5-phenylloxazolyl) benzene; PUFA — polyunsaturated fatty acid; ROS — reactive oxygen species.

stopped tube were heated, at 37 °C or 42 °C controlled within ± 0.05 °C for 1 h in a water bath (Model C-650, Taiyo Scientific Industrial Co., Ltd., Japan) [11].

Assay of DNA synthesis. DNA synthesis was assayed by monitoring of [^3H]-thymidine (0.74–1.1 TBq/mmol, Amersham Japan, Tokyo) incorporated into the acid-insoluble fraction of cells. After heat treatment, cells were cultured at 37 °C for 19 h, nearly equal to the cell cycle period, or 96 h for long-term culture, and labeled with 1 μCi (37 kBq) of [^3H]-thymidine for 1 h. Then, cells were rinsed twice with physiological saline, rinsed with 5% trichloroacetic acid, dried, and placed in a counting vial containing 5 ml of a toluene-based scintillator liquid containing 0.4% diphenyloxazole (DPO), and 0.01% 1.4-bis-2-(4-methyl-5-phenyloxazolyl) benzene (POPOP). The radioactivity was measured with a liquid scintillation counter (Beckman Instrument, Inc., Irvine, CA).

Detection of EPA or DHA incorporated into cells. Cells at the final density of $5 \times 10^5/\text{ml}$ and EPA or DHA at 100 μM were mixed in the total volume of 80 ml in 4 glass tubes (32 mm i.d. x 78 mm). Cells were treated at 37 °C or 42 °C for 1 h, and immediately centrifuged. After the supernatant was removed, cell pellets were suspended in fresh culture medium, transferred into a centrifuge tube, and then rinsed twice by centrifugation at 3000 rpm for 10 min. Supernatant was withdrawn with a pipette. The resultant sediment was mixed with 0.5 ml chloroform-methanol (2 : 1, by volume), homogenized for 2 min, and centrifuged. Supernatant containing lipids was transferred into another glass tube. The solvent was evaporated in atmosphere of nitrogen gas. The extracted lipids were separated by a modified method for two-dimensional TLC on a silica gel plate and developed with two solvent mixtures [2, 13]. The TLC was developed with chloroform-methanol-water (65 : 25 : 4, V : V : V) [14] to separate polar lipids, and replaced by the first solvent mixture at this time, and nonpolar lipids such as fatty acids and triglycerides were separated with petroleum-ethylether-acetic acid (80 : 18 : 2) [15]. The plate was exposed to iodine and lipid fractions were detected. Silica gel portions containing fatty acids in the TLC plate were shaved, and the powder was transferred into a glass tube (5.5 mm i.d. x 200 mm length) sealed with cotton in the bottom. Then a mixture of chloroform-methanol (1 : 4, V : V) was poured into the tube, and the solvent was evaporated. The extracted lipids were hydrolyzed, and fatty acids were esterized with methanol containing 5% hydrochloride (Wako Pure Chemical Co., Japan). Methyl esters of EPA and DHA were analyzed with a gas chromatograph equipped with a flame ionization detector (Shimadzu Seisakusho Ltd. Kyoto). The column used was packed with diethyleneglycol succinate (10%, 1 m long). The column temperature was retained at 195 °C and the flow rate of carrier gas (nitrogen) was 60 ml/min [2].

Statistics. The statistical differences between the incorporation rates of [^3H]-thymidine in the presence

and absence of EPA or DHA at the temperatures of 37 °C, 40 °C, and 42 °C were analyzed using Student's *t*-test.

RESULTS

Inhibitory effect of EPA and DHA on DNA synthesis. DNA synthesis in Ehrlich ascites tumor (EAT) cells treated with EPA for 1 h and subsequently cultured for 19 h was inhibited in a manner dependent on EPA concentrations and treatment temperatures (Fig. 1). Upon the treatment with 50 μM EPA at 37 °C, the rate of [^3H]-thymidine incorporation was inhibited to $63.1 \pm 3.0\%$ ($P < 0.001$) compared to the control in the absence of the fatty acids, whilst treatment with 100 and 150 μM of EPA resulted in the inhibition to $36.7 \pm 2.4\%$ ($P < 0.001$) and $16.5 \pm 4.3\%$ ($P < 0.001$), respectively. At 40 °C, inhibition of [^3H]-thymidine incorporation in the absence of fatty acids was not altered compared to that at 37 °C, but if 50, 100, and 150 μM of EPA were added, the incorporations were inhibited to $59.9 \pm 3.7\%$ ($P < 0.001$), $29.0\% \pm 1.9\%$ ($P < 0.001$), and $8.3 \pm 1.4\%$ ($P < 0.001$), respectively. In contrast, heat treatment alone at 42 °C inhibited [^3H]-thymidine incorporation to $57.2 \pm 2.7\%$ ($P < 0.001$) compared to control EAT cells at 37 °C. At 42 °C the inhibition of DNA synthesis was also enhanced by EPA administration (to $41.5 \pm 2.0\%$ ($P < 0.001$), $33.1 \pm 1.4\%$ ($P < 0.001$), $19.7 \pm 1.2\%$ ($P < 0.001$), and $7.7 \pm 0.6\%$ ($P < 0.001$) for 25, 50, 100, and 150 μM EPA, respectively). Thus, the treatment with EPA at 42 °C resulted in a slightly higher inhibition (a 50% inhibitory concentration (IC_{50}), 66 μM) of DNA synthesis in tumor cells than that at 37 °C (IC_{50} , 77 μM), and was similar to that at 40 °C (IC_{50} , 67 μM).

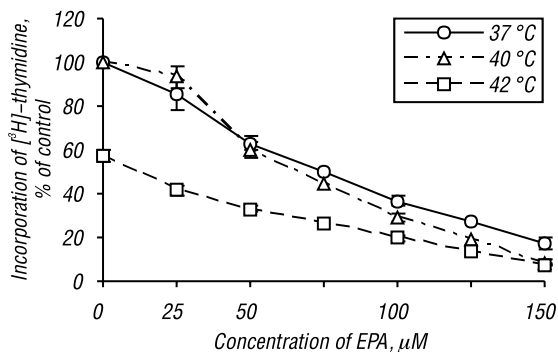


Fig. 1. Inhibitory effect of eicosapentaenoic acid (EPA) on DNA synthesis in Ehrlich ascites tumor cells. Cells were treated with EPA of indicated concentrations at 37 °C, 40 °C, or 42 °C for 1 h and subsequently cultured for 19 h. Cells (1×10^5 cells/ml) were then labeled with [^3H]-thymidine for 1 h. The incorporations into the control (untreated) cells were $40,652 \pm 2,356$ d.p.m. Values represent the mean \pm SEM for quadruplicate measurements and are shown as a percentage of incorporation in the absence of EPA at 37 °C (control)

Inhibitory effect of DHA on DNA synthesis in EAT cells treated in similar manner is shown in Fig. 2. At 37 °C DHA inhibited significantly [^3H]-thymidine incorporation into DNA of EAT cells to $87.9 \pm 6.1\%$ ($P < 0.05$), $32.7 \pm 3.5\%$ ($P < 0.001$), and $4.1 \pm 1.8\%$ ($P < 0.001$) compared to the control at 50, 100, and 150 μM of DHA, respectively. At 40 °C, the incorporation decreased to $67.9 \pm 2.8\%$ ($P < 0.001$), $19.2\% \pm 2.1\%$ ($P < 0.001$), and $0.5 \pm 0.1\%$ ($P < 0.001$). Based on a decrease in DNA synthesis

to $57.2 \pm 2.7\%$ ($P < 0.001$) by hyperthermia alone at 42°C , the inhibition rate increased upon combination of hyperthermia and DHA administration and yielded to $30.7 \pm 2.1\%$ ($P < 0.001$), $10.6 \pm 0.9\%$ ($P < 0.001$), and $0.9 \pm 0.5\%$ ($P < 0.001$) for 50, 100, and 150 μM DHA, respectively. Thus, a more marked inhibition (IC_{50} , 55 μM) of cellular DNA synthesis at 42°C than that at 37°C or 40°C (IC_{50} , 85 or 69 μM , respectively) was also observed for the treatment with DHA.

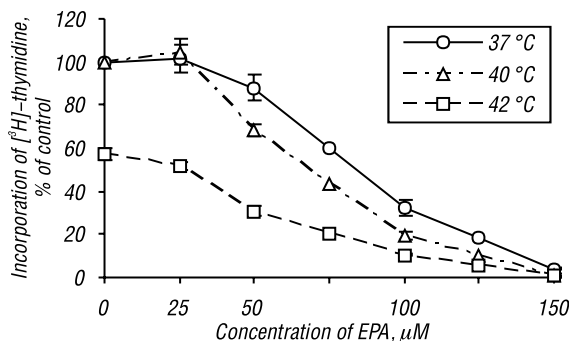


Fig. 2. Inhibitory effect of docosahexaenoic acid (DHA) on DNA synthesis in Ehrlich ascites tumor cells. Cells were treated with DHA at 37°C , 40°C , or 42°C and manipulated in the same manner as in Fig. 1. Values represent the mean \pm SEM for quadruplicate measurements and are shown as a percentage of incorporation in the absence of DHA at 37°C (control)

Next, DNA synthesis-inhibiting activities of EPA and DHA at lower or higher concentrations at three temperatures were compared (Fig. 3). The ratio of DNA synthesis values in EAT cells treated with DHA versus that with EPA under the same treatment conditions was calculated from data in Fig. 1 and Fig. 2. Note that a DNA synthesis-inhibiting activity of DHA is higher at 100 and 150 μM , but lower at 25 and 50 μM than that of EPA regardless of temperatures, but is more pronounced at higher temperatures.

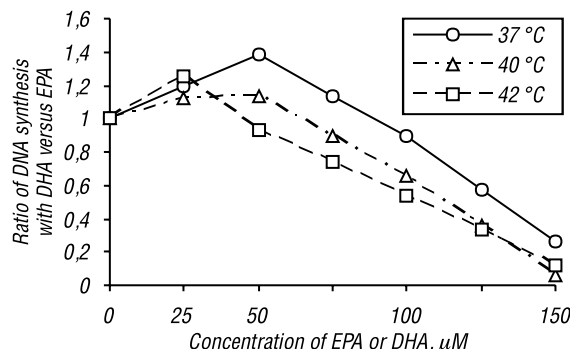


Fig. 3. Comparison of DNA synthesis-inhibiting activity of EPA and DHA at low or high concentrations at 37°C , 40°C , or 42°C . The ratio of DNA synthesis values in Ehrlich ascites tumor cells treated with DHA versus that with EPA under the same treatment conditions including concentrations and temperatures was calculated from data in Fig. 1 and Fig. 2. Note that a DNA synthesis-inhibiting activity of DHA is higher at 100 and 150 μM , but lower at 25 and 50 μM than that of EPA

Ratio of DNA synthesis in EAT cells exposed to EPA or DHA at 42°C versus that at 37°C was also calculated from data in Fig. 1 and Fig. 2 (Fig. 4). More enhanced combined effects of hyperthermia at 42°C were exhibited by DHA than by EPA at the doses higher than 50 μM .

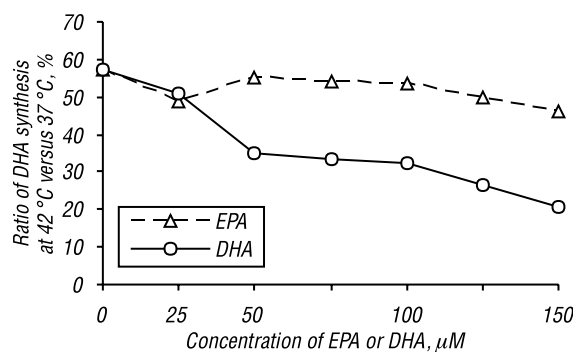


Fig. 4. Hyperthermic enhancement of inhibitory effects of EPA and DHA on DNA synthesis. Ratio of DNA synthesis in cells exposed to EPA or DHA at 42°C versus 37°C was calculated and illustrated

Compared to the above-mentioned treatment of EAT cells cultured for 19 h with two polyunsaturated fatty acids (PUFA), 5-fold longer culturing of cells upon the same treatment resulted in enhanced inhibition of DNA synthesis by EPA or DHA. Inhibitory effect of EPA or DHA on DNA synthesis in EAT cells treated at 37°C or 42°C for 1 h and then cultured for 96 h was shown in Fig. 5. Incorporation of $[^3\text{H}]$ -thymidine in the cells exposed to 25 and 50 μM EPA at 37°C decreased to $78.1 \pm 7.1\%$ ($P < 0.01$) and $0.5 \pm 0.1\%$ ($P < 0.001$) of the control level, respectively. Hyperthermia alone at 42°C decreased the rate of $[^3\text{H}]$ -thymidine incorporation to $87.6 \pm 1.8\%$ ($P < 0.02$) of the control value, which was higher than that for the short-term culture (57.2%). So, the long-term culturing leads to repair from hyperthermic injuries in the absence of EPA or DHA. In contrast, the inhibition rates were enhanced to $30.0 \pm 3.1\%$ ($P < 0.001$) and $0.6 \pm 0.3\%$ ($P < 0.001$) with 25 and 50 μM EPA, respectively. The values of $[^3\text{H}]$ -thymidine incorporation upon addition of 25 and 50 μM DHA at 37°C were $80.0 \pm 8.0\%$ ($P < 0.02$) and $11.3 \pm 4.7\%$ ($P < 0.001$) of the control, respectively, whilst at 42°C they decreased to $46.2 \pm 8.2\%$ ($P < 0.001$) and $4.5 \pm 2.1\%$ ($P < 0.001$), respectively. Thus, the long-term culturing intensified the inhibition of DNA synthesis by 50 or 75 μM EPA or DHA at both temperatures, but in contrast restored the DNA synthesis inhibition due to hyperthermia alone. It was suggested that hyperthermic injuries can be repaired whereas in combination with PUFA sublethal additional injuries appear which are enhanced to lethal injuries by the next long-term culturing.

Relation between the inhibitory activity of drugs and their permeation into cells.

Incorporation rates of EPA and DHA into EAT cells have been previously measured by TLC and gas-liquid chromatography (GLC) to find a relationship with the cytotoxic effects [2]. The fatty acids are incorporated mainly into phospholipids (PLs) and triglycerides (TGs) in the cells [2, 16–18]. Inhibition of DNA synthesis has been suggested to be due to their free acid forms accumulated in the cells as a result of the extracellular supply of PUFAs so abundant as to exceed beyond the cellular ability to synthesize PLs and TGs [2].

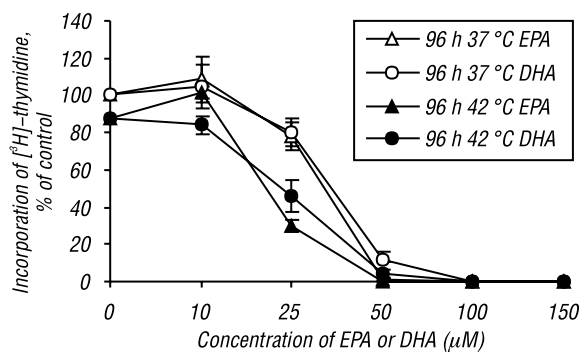


Fig. 5. Inhibitory effect of EPA or DHA on DNA synthesis in Ehrlich ascites tumor cells treated at 37 °C or 42 °C for 1 h and further cultured for a period as long as 96 h. Cells (1×10^4 cells/ml) were then labeled with [^3H]-thymidine for 1 h. The incorporations in the control (untreated) cells were $210,481 \pm 11,015$ d.p.m. Values represent the mean \pm SEM for quadruplicate measurements and are shown as a percentage of incorporation in the absence of EPA and DHA at 37 °C (control).

In the present study, therefore, contents of EPA and DHA in the free fatty acid (FFA) form incorporated into the cells exposed to 100 μM of each fatty acid were measured. Incorporation of DHA and EPA into lipids of cells are shown in thin-layer chromatograms (Fig. 6) and the spots of FFA and TG in the cells treated with 25 or 100 μM EPA or DHA were markedly larger, than these in the control cells cultured in the absence of EPA/DHA.

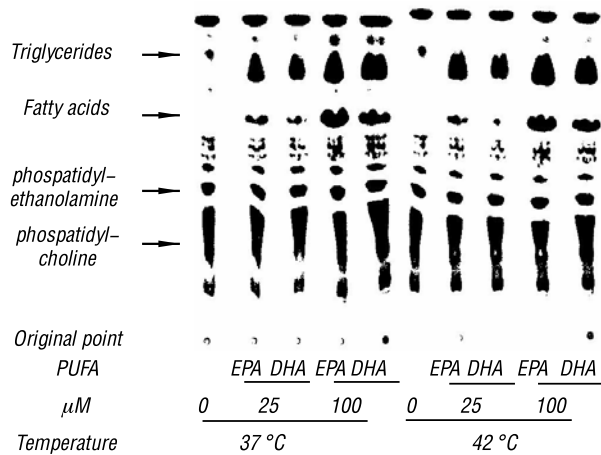


Fig. 6. Thin-layer chromatograms of lipids extracted from Ehrlich ascites tumor cells incubated with docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Cells (4×10^7 of total cells) at the density of 5×10^5 /ml were incubated with 100 μM of DHA or EPA at 37 °C or 42 °C for 1 h. Lipids containing the fatty acids incorporated into the cells were extracted. Two-dimensional thin-layer chromatography on a silica gel plate was developed with chloroform-methanol-water (65 : 25 : 4, by volume) to separate phospholipids and with petroleum ether-ethyl ether-acetic acid (82 : 18 : 1) to separate fatty acids, triglycerides etc. The plate was exposed to iodine and lipid fractions were detected

Content of DHA and EPA extracted from FFA fractions separated by TLC was quantified by gas-liquid chromatography (GLC) (respective gas-liquid chromatograms are shown in Fig. 7). Incorporation rate of EPA was 0.29% of the total dose and that of DHA was 0.97% at 37 °C. At 42 °C, the contents of EPA and DHA increased to 0.40% and 1.16%, respectively.

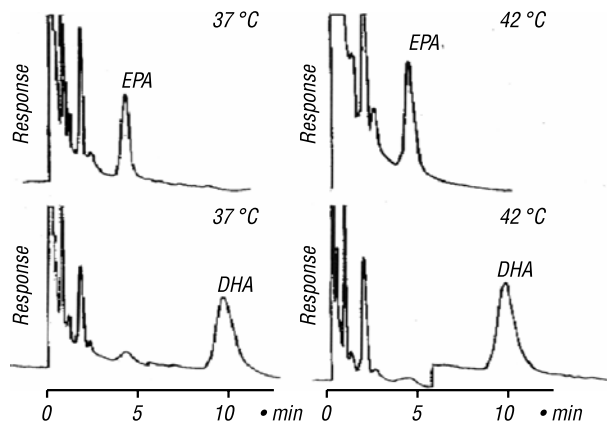


Fig. 7. Gas-liquid chromatograms of extracts from lipids of Ehrlich ascites tumor cells exposed to EPA and DHA at 37 °C or 42 °C for 1 h. Fatty acids were extracted from cellular FA fraction separated by TLC and hydrolyzed, and fatty acids were esterized with methanol containing 5% hydrochloride. Methyl esters of EPA and DHA were analyzed with a gas chromatograph equipped with a column containing diethyleneglycol succinate (10%, 1 m long). The column temperature was retained at 195 °C and the flow rate of carrier gas (nitrogen) was 60 ml/min

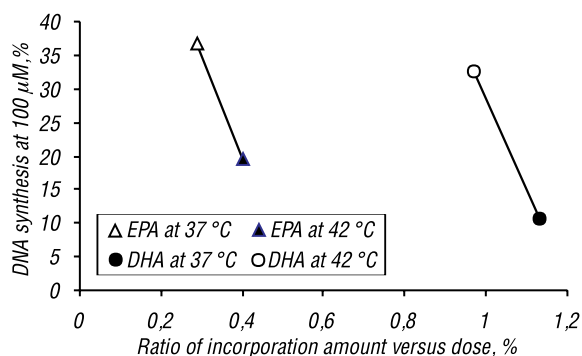


Fig. 8. A relation of DNA synthesizing activity and ratio of intracellular incorporation amount versus dose of EPA or DHA. Cells treated with 100 μM of EPA or DHA at 37 °C or 42 °C were incubated in a CO_2 incubator for 1 h. Intracellular EPA or DHA was extracted and quantified by TLC and GLC and evaluated as the ratio versus the extracellularly added amount. A correlation of DNA synthesizing activity and incorporation were plotted with their values

DISCUSSION

Cytotoxic or antitumor activity of PUFAs has been previously reported [1–6]. The activity depends on the length of carbon chain and the number of unsaturated bonds, and particularly elevates according to the increase in numbers of double bonds in PUFAs [2, 12]. We have reported that EPA and DHA having higher number of double bonds, exert stronger inhibition of the DNA synthesis in tumor cells than other PUFAs [2]. In the present study, furthermore, it was confirmed that at lower concentrations DHA inhibits the synthesis less markedly than EPA, in contrast to advantages at higher concentrations upon the treatment at 37 °C, 40 °C or 42 °C (Fig. 3). The results are supported with a difference of the binding ability of serum albumin to EPA and DHA [16], and serum albumin can bind more abundant DHA than EPA, whereas DHA is more effective than EPA at the lower concentrations under serum free conditions [2]. The unbound drugs that are exempted from capture by albumin may be subjected to uptake into cells. Then EPA is mainly

incorporated into TG [2, 17–19], and DHA to PC within the cell. Consequently, they lose cytotoxic activity assumedly because of esterification of the terminal carboxyl group in the molecule. Administration of the large amount of drugs overcomes both their binding activity to serum proteins and the cellular ability to synthesize the lipids. Thus, free forms of the fatty acids are accumulated in the cells and considered to exert a cytotoxic action. Intracellular accumulation of free DHA is more abundant than that of free EPA (Fig. 6). Their accumulation results in cytotoxic effects via production of reactive oxygen species (ROS) [20–22], that may be furthermore enhanced by combination with hyperthermia, and consequently conduct the promotion of cell-killing [23].

The present study showed that 50 μ M EPA or DHA markedly inhibited DNA synthesis in tumor cells cultured for 19 h after the treatment at 37 °C. Hyperthermic treatment enhanced the inhibition rate and the rate of inhibition of DNA synthesis was enhanced by long term culturing, during which a growing amount of the free acid form of PUFA is considered to be metabolized and then generate more abundant ROS.

A relation between intracellular contents of the free acid form of EPA and DHA and their inhibitory effect is shown. EPA exhibits higher inhibitory activity in spite of a lower content of incorporation in the cells at 37 °C or 42 °C, whereas ratio of the incorporation content versus the inhibitory activity for DHA is nearly 3-fold higher than that for EPA. This difference could be probably explained by the fact that EPA is more stable in an intracellular space and is less easily metabolized than DHA. This is supported by the fact that EPA is more cytotoxic than DHA on the long-term culturing conditions.

Among fatty acids EPA and DHA have the highest inhibitory activity against cellular DNA synthesis and such activity is significantly enhanced by hyperthermia. The inhibition of DNA synthesis is closely related to the viability of the cells [2]. Therefore, administration of EPA or DHA in combination with hyperthermia could be considered as an attractive mean for treatment of cancer.

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ГИПЕРТЕРМИЯ УСИЛИВАЕТ СТЕПЕНЬ ПОДАВЛЕНИЯ СИНТЕЗА ДНК ПРИ ОБРАБОТКЕ КЛЕТОК КАРЦИНОМЫ ЭРЛИХА ЭЙКОЗОПЕНТАЕНОВОЙ И ДОКОЗАГЕКСАЕНОВОЙ КИСЛОТАМИ

Цель: исследовать ингибирующее воздействие эйкозопентаеновой кислоты (ЕРА) и докозагексаеновой кислоты (ДНА) на синтез ДНК в комбинации с гипертермией в модели *in vitro*. **Методы:** суспензию клеток асцитной карциномы Эрлиха (ЕАТ) смешивали с ДНА или ЕРА в стеклянной пробирке, инкубировали в течение 1 ч при 37 °С, 40 °С или 42 °С в водяной бане и культивировали при 37 °С 19 или 96 ч. Синтез ДНК оценивали по уровню включения [³H]-тимидина. ДНА или ЕРА, включившиеся в клетки ЕАТ cells, экстрагировали, а их количество определяли методами тонкослойной и газожидкостной хроматографии. **Результаты:** степень ингибирования синтеза ДНК при действии ЕРА или ДНА значительно возрастала при обработке клеток при 42 °С в сравнении с таковым при 37 °С. При 37 °С ингибирующее действие ЕРА было более выраженным, чем таковое ДНА в низких концентрациях (при концентрации 50 мкМ — 63,1 против 87,9%), а ДНА — более сильным при концентрации 150 мкМ (16,7 vs 4,4%). Эффект ДНА в сравнении с ЕРА был более выраженным при 40 °С (29,0 vs 19,2% при концентрации 100 мкМ) или 42 °С (19,7 vs 10,6% при концентрации 100 мкМ). При обработке клеток ЕРА или ДНА с последующим культивированием в течение 96 ч наблюдалось усиление ингибирующего воздействия ЕРА даже при концентрации 50 мкМ как при 37 °С (0,5 vs 11,3%), так и при 42 °С (0,6 vs 4,5%), что в таких условиях было значительнее, чем у ДНА. В то же время степень включения ЕРА в клетки ЕАТ при 37 °С или 42 °С была ниже, чем таковая ДНА. **Выводы:** введение ДНА или ЕРА *in vitro* значительно ингибирует синтез ДНК, и этот эффект усиливается на фоне комбинированного применения кислот и гипертермии. **Ключевые слова:** эйкозопентаеновая кислота, докозагексаеновая кислота, гипертермия, цитотоксическая активность, синтез ДНК.