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The Effects of Parenteral Lipid Emulsions on Cancer and Normal Human Colon Epithelial Cells *in vitro*

J. HOFMANOVÁ[1], Z. ZADÁK[2], R. HYŠPLER[2], J. MIKESKA[3], P. ŽĎÁNSKÝ[2], A. VACULOVÁ[1], J. NETÍKOVÁ[1], A. KOZUBÍK[1]

[1]Laboratory of Cytokinetics, Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, [2]Department of Metabolic Care and Gerontology, Charles University, Teaching Hospital, Hradec Králové and [3]Infusia Hořátev, a. s., Czech Republic

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Summary

Differences in lipid metabolism of tumor and normal tissues suggest a distinct response to available lipid compounds. In this study, the *in vitro* effects of five types of commercial parenteral lipid emulsions were investigated on human cell lines derived from normal fetal colon (FHC) or colon adenocarcinoma (HT-29). Changes of the cellular lipid fatty acid content, cell oxidative response, and the cell growth and death rates were evaluated after 48 h. No effects of any type of emulsions were detected on cell proliferation and viability. Compared to the controls, supplementation with lipid emulsions resulted in a multiple increase of linoleic and linolenic acids in total cell lipids, but the content of arachidonic, eicosapentaenoic, and docosahexaenoic acids decreased particularly in HT-29 cells. The concentration of emulsions which did not affected HT-29 cells increased the percentage of floating and subG_0/G_1 FHC cells probably due to their higher reactive oxygen species production and lipid peroxidation. Co-treatment of cells with antioxidant Trolox reduced the observed effects. Our results imply that lipid emulsions can differently affect the response of colon cells of distinct origin.

Key words

Fat emulsion • Reactive oxygen • Lipid peroxidation • Apoptosis • Tumor cells

Introduction

Lipids in artificial nutrition play an important role in metabolic reactions and immunity of critically ill patients (Adolph 1999). Recently, it has become clear that besides direct nutritional effects, lipids play numerous structural and regulatory roles which may have an important impact on physiological functions in the organism. Different types of fatty acids may influence cell membrane fluidity, receptor mobility and functions, signal transduction as well as eicosanoid synthesis and cytokine production and functions (Grammatikos *et al.* 1994a, Calder 2001, Hong *et al*. 2002). Lipid mediators have a potent impact on a wide variety of cellular responses including cell growth, differentiation and apoptosis (Maziere *et al.* 1999, Rudolph *et al.* 2001). Certain polyunsaturated fatty acids (PUFAs) were found to inhibit tumor growth and cancer cachexia (Tisdale and Dhesi 1990, Petrik *et al.* 2000). Recruitment of specific lipids by tumor cells made them more sensitive to

specific endogenous regulators or antitumor agents (Heys *et al.* 1996). Therefore, lipid emulsions containing PUFAs as essential components should be considered not only as a source of energy but also as a tool for specific pharmacological targeting. Lipids in "disease-specific" artificial nutrition could influence the course of many pathological reactions including tumor growth and the metastatic potential. Application of lipid emulsion can also reduce toxicity of drugs (Červinková and Drahota 1998). However, little is known about the effects of lipid emulsions on specific cell types and about differences in the response of fetal and adult normal or cancer cells. Differences in lipid metabolism of tumors and normal tissues (Spector and Burns 1987, Cheeseman *et al.* 1988) suggest a distinct response to supplied lipid compounds and imply the possibility for adjuvant anticancer therapy using PUFAs.

Therefore, we made *in vitro* experiments investigating the effects of five different types of parenteral lipid emulsions based on either soya bean oil with long-chain triglycerides (LCT) or mixed LCT and medium-chain triglycerides (LCT/MCT) currently used in clinical practice. Changes of the fatty acid content of cellular lipids, oxidative response and parameters reflecting cell growth and death were evaluated using human cells originating from colon adenocarcinomas or normal fetal colon tissue.

Material and Methods

Cell cultures

The human colon adenocarcinoma HT-29 cells (HTB 38; ATCC, Rockville, MD) were cultured in McCoy's 5A medium supplemented with 50 μg/ml gentamicin (Sigma-Aldrich, Prague, Czech Republic) and 10 % fetal calf serum (FCS, PAN Biotech GmbH; Aidenbach, Germany). The normal human fetal colon FHC cells (CRL-1831; ATCC, Rockville, MD) were cultured in a 1:1 mixture of Ham's F12 and Dulbecco's modified Eagle's mediums containing HEPES (25 mM), cholera toxin (10 ng/ml), insulin (5 μg/ml), transferrin (5 μg/ml) and hydrocortisone (100 ng/ml) (Sigma-Aldrich, Prague, Czech Republic), and supplemented with 10 % FCS. The cultures were maintained at 37 °C in 5 % CO_2 and 95 % humidity.

Lipid emulsions

Five commercially produced lipid emulsions Nutralipid Infusia MCT 20 %, Nutralipid Infusia P 20 % (Infusia Hořátev, a.s., Czech Republic), Intralipid 20 % (Fresenius Kabi AB, Uppsala, Sweden), Lipofundin MCT/LCT (B. Braun, Melsungen AG, Germany), and Elolipid 20 % (Fresenius, Pharma, Austria) were used. Composition of emulsions is described in Table 1. Sterile emulsions were applied to the cells (24 or 72 h after seeding) diluted at the rate of 1:50 - 1:3200 in the cultivation media. Thus the proportion of originally 20 % oil fraction was 0.4-0.006 %. For comparison of the effects on HT-29 and FHC cells emulsions diluted at the rate of 1:400 (0.05 %) were used. The final concentration of FCS during the experiments was 5 %. Antioxidant Trolox (water soluble derivative of α-tocopherol, 50 μM, Sigma-Aldrich, Prague, Czech Republic) was added to the cells simultaneously with the emulsions.

Table 1. Composition of parenteral lipid emulsions (in grams) tested using *in vitro* system.

Components	Intralipid	Elolipid	Lipofundin MCT/LCT	Nutralipid MCT	Nutralipid P
Soybean oil	200	200	100	100	200
Egg phospholipids	12	12	12	12	12
Glycerol	22	25	25	22.5	22.5
Coconut oil (MCT)	–	–	100	100	–
α-tocopherol	–	–	0.2	–	–
Sodium oleate	–	–	0.3	–	–
Oleic acid	–	0.30 - 0.45	–	–	–
Sodium hydroxide	to pH 8	0.04 - 0.08	–	–	–
Water for injection to	1 000	1 000	1 000	1 000	1 000

Data supplied by the manufacturers.

Analysis of fatty acid composition

The content of individual fatty acids in the fresh cell-free cultivation medium supplemented with diluted (1:400) lipid emulsions (prior to addition to the cells) as well as in total lipids of cells treated with the emulsions for 48 h was detected. Aliquots (1 ml) of cell-free media with diluted emulsions were frozen in amber vials in –80 °C. Cells cultivated with emulsions were washed twice with phosphate-buffered saline (PBS), resuspended in a freezing medium (cultivation medium containing 10 % FCS and 5 % dimethylsulfoxide) and aliquots were frozen at –80 °C. After thawing total lipids were extracted from the samples and the fatty acid content was determined after transmethylation using a GC-MS TurboMass instrument (Perkin-Elmer, Norwalk, USA). The derivatization procedure was performed as published previously (Liebich *et al.* 1991). The content of individual fatty acids was standardized as nmol per 10^6 cells.

Production of reactive oxygen species (ROS)

Intracellular production of ROS was assayed by flow cytometric analysis using dihydrorhodamine-123 (DHR-123, Fluka, Switzerland), which reacts with intracellular hydrogen peroxide. Cells were washed twice in PBS, resuspended in Hank's buffered saline solution (HBSS) and incubated with DHR-123 (0.2 μM) at 37 °C and 5 % CO_2 for 15 min. Fluorescence was measured using a flow cytometer (FACSCalibur, Becton Dickinson, San Jose, CA) equipped with an argon laser at 488 nm wavelength for excitation and detected with a 530/30 (FL-1) optical filter. The acquired data were evaluated as the fluorescence of living cells using CellQuest software (Becton Dickinson).

Lipid peroxidation

The level of lipid peroxidation in the cells was measured by the spectrophotometric thiobarbituric acid (TBA, Sigma-Aldrich, Prague) assay as previously described (Canuto *et al*. 1995) with some modifications. Briefly, 0.75 ml of the cell suspension in PBS was added to the TBA reagent (0.5 ml of 15 % trichloroacetic acid; 0.5 ml of 0.25 N hydrochloric acid; 0.5 ml of 0.6 % TBA), incubated at 90 °C for 45 min, then cooled, extracted with 2.25 ml of N-butanol, and centrifuged (5 min, 1500 x *g*). The absorbance of the upper phase was measured at 532 nm. The concentration of thiobarbituric acid reactive substances (TBARS) was calculated from a standard calibration curve generated with known amounts of 1,1,3,3-tetraethoxypropane.

Cell number and viability assays

Floating and adherent cells were counted separately using a Coulter Counter (model ZM, Coulter Electronics, UK), and the number of floating cells was expressed as the percentage of the total cell number. Cell viability was determined by eosin (0.15 %) dye exclusion assay.

Cell cycle analysis

Floating and adherent cells were harvested together, washed in PBS, and fixed in 70 % ethanol at 4 °C. After washing with PBS, low-molecular-weight fragments of DNA were extracted for 10 min in citrate buffer (Na_2HPO_4, $C_6H_3O_7$, pH 7.8), RNA was removed by ribonuclease A (5 Kunitz U/ml), and DNA was stained with propidium iodide (20 μg/ml PBS) for 30 min in the dark. A total of $2x10^4$ cells were analyzed by flow cytometry in each sample. The ModFit 2.0 (Verity Software House, Topsham, ME) and CellQuest software was used to generate DNA content frequency histograms and quantify the amount of the cells in the individual cell cycle phases including the $subG_0/G_1$ population (reflecting apoptotic cells).

Statistical analysis

The results of at least three independent experiments were expressed as the means ± S.E.M. Statistical significance ($P<0.05$) was determined by one-way ANOVA followed by Tukey or LSD tests or by non-parametric Kruskal-Wallis or Mann-Whitney tests.

Results

Effects of lipid emulsions on colon cancer HT-29 cell growth and death

The total cell numbers (floating and adherent cells together) and viability of HT-29 cells treated with emulsions (diluted 1:50-1:3200) for 48 h were not significantly different from untreated controls (data not shown). However, at higher concentrations of emulsions (diluted 1:50 and 1:100) a significantly increased percentage of floating cells was observed in comparison with untreated controls. The percentage of the floating HT-29 cells treated with both types of Nutralipid was about 2-3 times lower than after treatment with Intralipid, Elolipid or Lipofundin which caused about 30-40 % of floating cells (Table 2).

The cell cycle analysis showed only non-significant differences in the number of HT-29 cells in individual cell cycle phases after treatment with

emulsions as compared to the controls (data not shown). However, Lipofundin significantly decreased the amount of HT-29 cells in S-phase (by about 5 % compared to the controls) simultaneously increasing the G_0/G_1 phase (by about 7 % compared to the controls).

Table 2. The effects of various concentrations of lipid emulsions on percentage of floating HT-29 cells after 48 h of treatment.

Emulsion	Dilution	% of floating cells
None (control)	0	5.1±0.9 ^{x}
Intralipid	1:50	39.2±9.1 ^{x}
	1:100	34.9±6.1 ^{x}
	1:400	11.9±1.0 ^{x}
Elolipid	1:50	35.2±7.5 ^{x}
	1:100	34.9±6.4 ^{x}
	1:400	11.9±3.8
Lipofundin MCT/LCT	1:50	30.1±8.0 ^{x}
	1:100	14.4±3.4 ^{x}
	1:400	5.6±0.7
Nutralipid MCT	1:50	13.5±1.0 *
	1:100	9.0±0.9 *
	1:400	5.3±1.7
Nutralipid P	1:50	20.5±3.4 *
	1:100	1.1±0.8 *
	1:400	6.4±0.9

The numbers are the means ± S.E.M. of three independent experiments. $P<0.05$, * Tukey or LSD tests, ^{x} non-parametric Kruskal-Wallis test, compared to controls.

Comparison of the effects of lipid emulsions on HT-29 and FHC cells

The effects on changes of fatty acid content in total cell lipids, oxidative metabolism and cell growth and death of colon cancer HT-29 and normal fetal colon FHC cells were compared. For these experiments, the first from concentration not significantly affecting HT-29 cytokinetics was chosen, i.e. the dilution 1:400, which corresponds to 0.05 % solution of oil fraction (20 %) in emulsion.

Fatty acid composition of cellular lipids

In both HT-29 and FHC cells cultivated in media supplemented with emulsions for 48 h, the content of representative fatty acids in total cell lipids was affected in comparison with untreated controls. However, the pattern of this modulation was rather different between the two cell lines (Table 3). The amount of linoleic acid (LA, 18:2, n-6) was markedly increased after treatment with all types of lipid emulsions compared to the controls in HT-29 as well as FHC cells. The amount of linolenic acid (LNA, 18:3, n-3) was increased only in HT-29 cells. On the other hand, the content of desaturation and elongation products of LA or LNA, especially arachidonic (AA, 20:4, n-6), eicosapentaenoic (EPA, 20:5, n-3) and docosahexaenoic (DHA, 22:6, n-3) acids was decreased particularly in HT-29 cells, where the values represented only about 20 % of the controls after treatment with Intralipid or Elolipid. This decrease was less pronounced after treatment with Lipofundin (LCT/MCT).

Cell oxidative metabolism

HT-29 and FHC cells were treated with emulsions 24 h after seeding and lipid peroxidation and ROS produced by the cells were measured after 48 h of cultivation with the emulsions. Lipid peroxidation (TBARS production per one million cells) tended to be higher in both HT-29 and FHC cells treated with emulsions than in control cells and it was more pronounced in FHC cells (Fig. 1). However, due to the high scatter of the values, significant effect was confirmed only after treatment with Elolipid and Lipofundin for HT-29, and Elolipid and Intralipid for FHC cells. Co-treatment of the cells with Trolox apparently suppressed lipid peroxidation.

Compared to non-treated controls, significant increase of ROS production which was reduced by co-treatment with Trolox was detected in both HT-29 and FHC cells after treatment with all types of emulsions. As far as lipid peroxidation is concerned, these effects were more pronounced in FHC cells (Fig. 2).

Cell growth and death

In HT-29 cells no effects of emulsion treatment (1:400 dilution) on the percentage of floating cells (Table 2), cell number, cell cycle, viability and percentage of $subG_0/G_1$ population were detected (data not shown). On the other hand, this concentration of emulsions, except for both types of Nutralipid, significantly increased the percentage of floating cells as well as the $subG_0/G_1$ population in FHC cells (Table 4). This response was reduced by co-treatment of cells with antioxidant Trolox. The total cell number, cell cycle parameters and cell viability were not significantly influenced (data not shown).

Table 3. The amount of representative fatty acids in HT-29 and FHC cell lipids after 48 h of treatment with lipid emulsions.

Cell line	Emulsion (1:400)	Fatty acid (nmol/million cells)				
		16:0	**18:1**	**18:2 (n-6)**	**18:3 (n-3)**	**20:3 (n-6)**
HT-29	*none (control)*	196.1±15.6	59.0±5.8	11.4±1.3	1.9±0.7	5.2±0.2
	Intralipid	21.2±0.4	2.8±0.5	5.7±0.2	0.4±0.1	20.7±1.2
	Elolipid	12.0±0.5	1.8±0.3	1.2±0.1	0.4±0.1	9.8±0.5
	Lipofundin MCT/LCT	8.2±0.3	5.9±0.7	2.6±0.4	0.6±0.2	7.3±0.3
	Nutralipid MCT	10.7±0.4	3.2±0.8	1.7±0.2	0.3±0.1	7.1±1.0
	Nutralipid P	13.9±1.3	2.6±0.3	1.3±0.1	0.6±0.1	8.3±0.2
FHC	*none (control)*	10.8±1.7	5.8±0.7	2.0±0.1	0.3±0.1	5.1±0.3
	Intralipid	128.8±12.3	59.1±4.3	21.8±1.2	1.4±0.3	1.2±0.1
	Elolipid	160.0±25.7	53.3±9.0	15.9±0.7	2.2±0.2	1.2±0.1
	Lipofundin MCT/LCT	150.6±14.4	62.2±6.3	23.9±0.7	1.3±0.1	2.1±0.2
	Nutralipid MCT	129.8±17.7	54.4±7.8	19.0±0.8	1.2±0.1	1.2±0.1
	Nutralipid P	151.8±23.4	61.2±9.2	20.2±1.1	3.0±0.4	1.2±0.2

Cell line	Emulsion (1:400)	Fatty acid (nmol/million cells)				
		20:4 (n-6)	**22:4 (n-3)**	**20:5 (n-3)**	**22:5 (n-3)**	**22:6 (n-3)**
HT-29	*none (control)*	37.9±1.4	2.6±0.2	8.7±0.3	0.4±0.1	20.3±0.6
	Intralipid	6.5±0.4	4.9±0.4	1.6±0.1	0.2±0.1	5.2±0.4
	Elolipid	5.7±0.4	4.8±0.3	1.7±0.1	0.1±0.1	5.0±0.4
	Lipofundin MCT/LCT	21.6±1.0	1.8±0.1	4.2±0.1	0.3±0.1	13.1±0.5
	Nutralipid MCT	14.9±0.9	1.8±0.2	2.8±0.1	0.6±0.1	8.1±0.5
	Nutralipid P	14.4±0.6	2.6±0.1	1.7±0.3	0.1±0.1	8.1±1.3
FHC	*none (control)*	21.2±0.4	2.8±0.5	5.7±0.2	0.4±0.1	20.7±1.2
	Intralipid	12.0±0.5	1.8±0.3	1.2±0.1	0.4±0.1	9.8±0.5
	Elolipid	8.2±0.3	5.9±0.7	2.6±0.4	0.6±0.2	7.3±0.3
	Lipofundin MCT/LCT	10.7±0.4	3.2±0.8	1.7±0.2	0.3±0.1	7.1±1.0
	Nutralipid MCT	13.9±1.3	2.6±0.3	1.3±0.1	0.6±0.1	8.3±0.2
	Nutralipid P	10.8±1.7	5.8±0.7	2.0±0.1	0.3±0.1	5.1±0.3

The numbers are the means ± S. E. M. of two independent experiments analyzed by HPLC in duplicates.

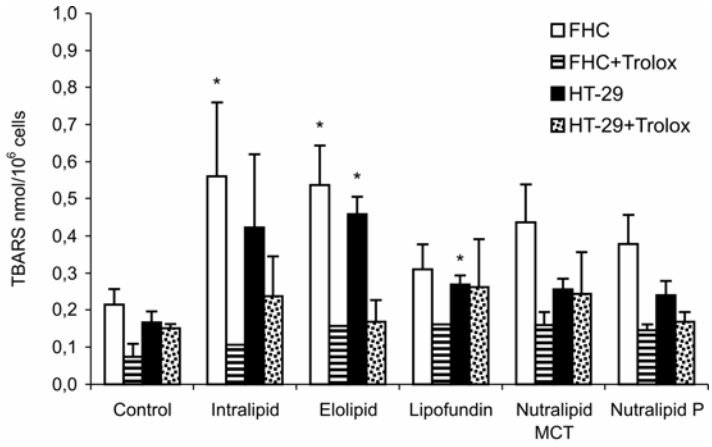

Fig. 1. Lipid peroxidation (thiobarbituric acid reactive substances – TBARS per million cells) of HT-29 and FHC cells after 48 h of treatment with lipid emulsions (diluted 1:400) with or without Trolox (50 μM). Values are means ± S.E.M of three independent experiments. Statistical significance: * P<0.05, compared to non-treated controls

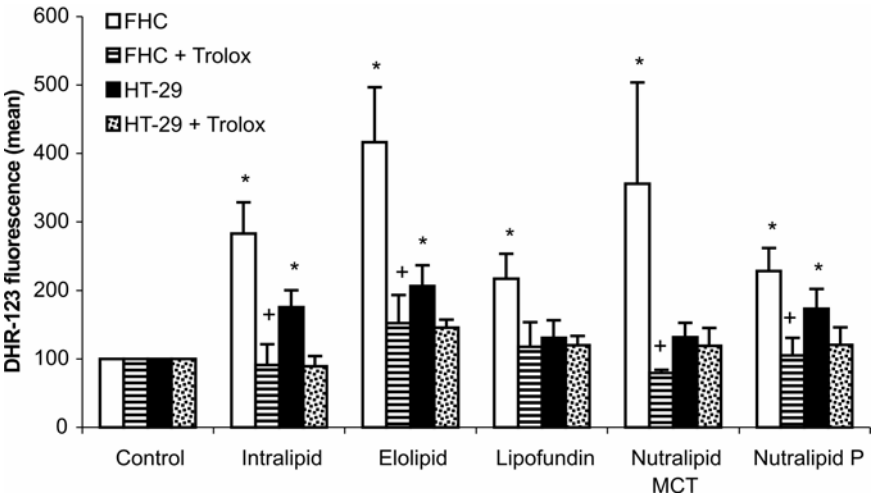

Fig. 2. Production of reactive oxygen species measured by flow cytometry as means of DHR-123 fluorescence (FL1-H) in HT-29 or FHC cells after 48 h of treatment with lipid emulsions (diluted 1:400) with or without Trolox (50 μM). Values expressed as percentage of control are means ± S.E.M of three independent experiments. Statistical significance: $P<0.05$, * compared to non-treated controls; $^{+}$ compared to appropriate samples without Trolox.

Table 4. The effects of lipid emulsions (diluted 1:400) and their combination with Trolox (50 μM) on total FHC cell number, viability, floating cells, and sub G_0/G_1 population after 48 h of treatment.

Emulsion	Trolox	Total cell number (milions)	Viability (%)	Floating cells (%)	Sub G_0/G_1 (%)
None (control)	–	1.47 ± 0.13	89.00 ± 4.22	6.78 ± 1.03	14.71 ± 1.85
	+	1.78 ± 0.15	92.25 ± 3.33	6.20 ± 1.02	19.61 ± 2.53
Intralipid	–	1.89 ± 0.12	83.33 ± 5.78	20.09 ± 1.56 $^{*+}$	43.46 ± 6.70
	+	1.89 ± 0.25	86.33 ± 4.26	5.98 ± 0.84	21.27 ± 6.65
Elolipid	–	1.48 ± 0.17	79.33 ± 2.40	21.96 ± 2.60 $^{*+}$	52.58 ± 8.81
	+	1.85 ± 0.12	89.33 ± 2.60	10.09 ± 0.15 ^{x}	15.83 ± 2.13
Lipofundin MCT/LCT	–	1.32 ± 0.12	85.33 ± 2.33	9.51 ± 0.34	37.87 ± 1.78
	+	1.46 ± 0.09	88.00 ± 1.53	15.59 ± 5.35 ^{x}	19.36 ± 0.27
Nutralipid MCT	–	1.41 ± 0.11	86.25 ± 2.39	8.34 ± 2.38	26.33 ± 4.55
	+	1.27 ± 0.12	88.50 ± 0.65	9.94 ± 1.79	19.80 ± 2.52
Nutralipid P	–	1.25 ± 0.13	87.25 ± 1.49	9.01 ± 0.80	30.53 ± 5.95
	+	1.86 ± 0.26	85.75 ± 5.66	5.40 ± 0.45	24.88 ± 4.42

The numbers are the means ± S. E. M. (in parenthesis) of three independent experiments. $P < 0.05$, (*) Tukey or non-parametric tests (Kruskal-Wallis and Mann Whitney) compared to appropriate control (^{x}) or to an appropriate sample with Trolox ($^{+}$).

Discussion

In our experiments, the amount of linoleic acid was increased in both HT-29 and FHC cell lipids after treatment with lipid emulsions. Moreover, the content of linolenic acid was also increased in HT-29 cells. This could be expected, because in cultivation media with diluted emulsions only these two types of PUFAs were detected (not shown). LA and LNA do not seem to be further metabolized in the cells because the content of

their elongation and desaturation products in cellular lipids was generally lower than in non-treated control. This is in agreement with experimental and clinical studies *in vivo* which demonstrated rapid incorporation and accumulation of the fatty acids from lipid emulsions into cell membrane phospholipids (Simoens *et al.* 1995), but a decreased content of long-chain AA and DHA (Dahlan *et al.* 1992). These effects could be avoided by using mixed LCT/MCT emulsions. The tendency to higher amounts of AA, EPA or DHA after LCT/MCT treatment in comparison with LCT was also observed in HT-29 cells in our *in vitro* system. It has been shown that LNA, especially in the presence of large amounts of LA, is not a very effective precursor for the formation of EPA and DHA in man and that cancer epithelial cells were not able to desaturate LA or LNA to AA, EPA or DHA (Grammatikos *et al.* 1994b).

Experimental studies have suggested a significant association between the high intake of n-6 PUFAs, especially LA, in Western diets and colorectal cancer risk (Singh *et al.* 1997, Galli and Marangoni 1997). On the other hand, fish oil (containing n-3 EPA and DHA) and olive oil (containing mostly oleic acid) were found to have protective effects (Anti *et al.* 1994, Stoneham *et al.* 2000). Lipid emulsion Omegaven containing fish oil inhibited growth and induced apoptosis of colon cancer Caco-2 cells *in vitro* (Jordan and Stein 2003). From this point of view, the results of our experiments documenting an increase of LA in colon epithelial cells treated with emulsions point to possible promoting effects on cancer cells. To clarify these effects more precise studies using other types of emulsions, e. g. containing n-3 PUFAs, are necessary.

In our experiments, the supplementation with any type of emulsion did not significantly affect cell proliferation (total cell number, cell cycle) and viability. The only effects of Lipofundin on the cell cycle may be explained by its different composition (LCT/MCT and presence of a higher amount of α-tocopherol). Similarly, it was shown that DNA synthesis in the rat regenerating liver was differently influenced by enteral feeding with LCT, MCT or their mixture (Bláha *et al.* 1999).

Our results have demonstrated that treatment with emulsions influenced cell adhesive properties enhancing the shedding of cells into the media. The type of detachment could be analogous to "anoikis", i.e. induction of apoptosis in response to loss of cell contact, typical of exfoliating epithelial cells (Frisch and Screaton 2001). This effect was stronger in FHC cells which significantly responded to emulsion concentrations which did not influenced HT-29 cells, e. g. dilution 1:400. The floating FHC cells were predetermined to apoptosis as demonstrated by the increased amount of cells with fragmented DNA detected in $subG_0/G_1$ population. Cells seem to be more sensitive to Elolipid and Intralipid than to Lipofundin as well as to both types of Nutralipid. In spite of the increased percentage of floating cells and $subG_0/G_1$ population, morphologically detectable apoptosis (in FHC cells stained with fluorescent dye DAPI) was low and non-significant in comparison with the controls (not shown) probably due to the relatively short time of cell cultivation.

We suggest that the stronger response of FHC cells compared to HT-29 cells could be conditioned by higher oxidative stress (ROS production and lipid peroxidation) evoked probably by PUFAs (Hofmanová *et al.* 2005) in the emulsions. This observation is supported by the fact that addition of antioxidant Trolox decreased the oxidative response as well as the percentage of floating and $subG_0/G_1$ cells. This implies the important role of oxidative metabolism in cell adhesive properties and induction of apoptosis after treatment of cells with lipid emulsions. It has been recognized that oxidants can serve as important mediators of specific cellular and molecular responses and gene expression (Aw 1999). Abnormal lipid composition (lower phospholipid content and low levels of PUFAs), a low level of cytochrome P450 enzymes, which can initiate and propagate lipid peroxidation (Cheeseman *et al.* 1988), and a higher level of the antioxidant system (Ozdemirler *et al.* 1998) in cancer cells compared to their normal counterparts can be associated with an increased rate of cell division and resistance to apoptosis (Gonzalez 1995). It was reported that in cell lines *in vitro* sensitivity to PUFAs also depends on their antioxidant defence mechanisms as well as culture conditions (Nøding *et al.* 1998).

Many reports and excellent reviews about the activities of PUFAs of n-3 and n-6 series in inflammation and cancer have been published (Grammatikos *et al.* 1994a, Calder 2001, Reddy 2004). Different lipid metabolism of the normal and tumor cells could produce selective effects of PUFAs (Grammatikos *et al.* 1994b). Modification of cellular functions by dietary lipids with a balanced ratio of n-6 and n-3 PUFAs offers an attractive opportunity to correct many pathophysiological processes (Koller *et al.* 2003) and to modify the effects of drugs (Plumb *et al.* 1993).

From another point of view, the different

response of FHC cells could be associated with their fetal tissue origin. Several reports have pointed out the importance of fat and differences in lipid metabolism during fetal and neonatal periods (Koldovský *et al.* 1995a, 1995b, Magnusson *et al.* 1997). Optimal composition of lipid emulsion for pregnant women and neonates has been investigated (Rubin *et al.* 1994, Jasnosz *et al.* 1995). Attention is paid particularly to adverse effects of oxidative stress due to reduced antioxidative defence mechanisms of infants (Pitkänen 1992) and to application of α-tocopherol (Linseisen *et al.* 2000). Our experiments proved that oxidative processes take place not only in the emulsion itself but also in the treated cells and these processes could influence cell behavior.

In summary, our results imply that currently used commercial lipid emulsions can affect the properties of colon epithelial cells (fatty acid composition, oxidative metabolism, cell adherence and induction of apoptosis). These changes depend on the composition of individual emulsions and can have a different impact in cells of distinct origin. Thus, these findings may contribute to a better understanding of the effects of lipid emulsions on specific cell types and may help in the deliberation of novel "disease-specific" nutrition.

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Reprint requests

A. Kozubík, Institute of Biophysics, Academy of Sciences of the Czech Republic, CZ-612 65 Brno, Královopolská 135, Czech Republic. Fax: +420541211293. E-mail: kozubik@ibp.cz