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Fatty acids decrease catalase activity in human leukaemia cell lines

Anna K. Azevedo-Martins [1]* and R. Curi [2]

[1] *School of Arts, Sciences and Humanities, University of São Paulo, São Paulo, Brazil*
[2] *Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil*

Fatty acid (FA) may disturb the redox state of the cells not only by an increase in reactive oxygen species (ROS) generation but also due to a reduction in antioxidant enzyme activities. The effect of various FAs (palmitic, stearic, oleic, linoleic, γ-linolenic and eicosapentaenoic acids (EPAs)) on Jurkat and Raji cells, (human T and B leukaemic cell lines was investigated). The following measurements were carried out: FA composition of the cells, cell proliferation and activities of catalase, glutathione peroxidase (GPx) and superoxide dismutase (SOD). The protective effect of α-tocopherol on cell death was also investigated. Each cell line presented a specific FA composition. All the tested FAs reduced catalase activity. The toxic effect of FA was abolished by the pre-incubation with physiological concentrations of α-tocopherol. The findings support the proposition that the increase in oxidative stress induced by FA partially occurs due to a reduction in catalase activity. In spite of the decrease in the enzyme activity, catalase protein and mRNA levels were not changed, suggesting a post-translational regulation. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS — fatty acids; lymphocytes; Jurkat cells; Raji cells; redox status; antioxidant enzymes; catalase

ABBREVIATIONS — PA, palmitic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid; LNA, γ-linolenic acid; EPA, eicosapentaenoic acid; FA, fatty acids; SOD, superoxide dismutase; GPx, glutathione peroxidase; HPLC, high performance liquid chromatography

INTRODUCTION

Fatty acids (FAs) modulate leukocyte function by controlling proliferation, cytokine production and synthesis of adhesion molecules.[1–6] Besides these effects, high concentrations of certain FA, particularly polyunsaturated fatty acids (PUFA), can cause cell death via apoptosis or, when concentrations are even higher, necrosis.[7,8] Cell death occurs by apoptosis at doses close to the physiological free FA concentration, as assessed by induction of internucleosomal DNA cleavage, chromatin condensation and nuclear breakdown.[7,9–11] High doses of FA preferentially cause necrosis, with a rapid loss of membrane integrity, lysosomal mediators leakage and cell swelling.[12] Both effects seem to be associated with oxidative stress, since they can be partially prevented by antioxidant compounds such as tocopherol.[7,13] Oxidative stress induced by FA may occur by an increase in reactive oxygen species (ROS) production and/or decrease in antioxidant enzyme activities. In this study some aspects of the toxic effects of FAs and the mechanisms involved were investigated in Jurkat and Raji cells, human T and B leukaemic cell lines, respectively. The following measurements were carried out: FA composition of the cells, cell proliferation and death and activities of catalase, glutathione peroxidase (GPx) and superoxide dismutase (SOD). The protective effect of α-tocopherol on cell death was also investigated. We postulate that FA may disturb the

* Correspondence to: Dr A. K. Azevedo-Martins, Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 1524, CEP: 05508-900 Butantan, São Paulo-SP, Brazil. Tel: (55) 11 3091 7245. Fax: (55) 11 3091 7285. E-mail: karenina@usp.br

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redox state of the cells not only by an increase in ROS production but also by a reduction in antioxidant enzyme activities.

MATERIALS AND METHODS

Cell culture conditions

Jurkat (human T leukaemic cell line) cells were obtained from the cell bank of the Dunn School of Pathology (Oxford University, UK) and Raji (human B leukaemic cell line) cells were obtained from the Rio de Janeiro Cell Bank (Rio de Janeiro, Brazil). Cells were grown in RPMI-1640 medium supplemented with 2 mM glutamine, 20 mM Hepes, 10% foetal calf serum (FCS), 10 U ml^{-1} penicillin G and 10 $\mu g\,ml^{-1}$ streptomycin. The cell population was maintained between 0.1 and 1.0×10^6 cells per ml (logarithmic phase of cell growth).

Fatty acid treatment

The FAs were diluted in ethanol and stored at $-70^{\circ}C$. The ethanol concentration in the medium was always lower than 0.5% of the final volume of cell suspension. The cells were exposed to different concentrations (0.05–0.2 mM) of the following FAs: palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), γ-linolenic (C18:3) and eicosapentaenoic (EPA, C20:5) acids. The procedure was similar to that used in our previous studies.[11] After 24 h culture, cell viability was determined. To examine the involvement of oxidative stress, cells were also pre-treated with α-tocopherol at 40 μM (final concentration) for 30 min before treatment with FAs.

Cell viability

Cell membrane integrity was assessed by the Trypan blue exclusion assay (0.5% Trypan blue in PBS). At least 100 cells were counted per sample.

Determination of fatty acid composition by high performance liquid chromatography (HPLC)

The FAs were extracted from Jurkat and Raji cells and from the culture medium as previously described.[14,15] After extraction and saponification,[16–18] the FAs were derivatized with 4-bromomethyl-7-coumarin[19] and the analysis performed in a Shimadzu model LC-10A liquid chromatograph (Japan). The samples were eluted using a C8 column (25 cm × 4.6 i.d., 5 μm particles) with pre-column C8 (25 cm × 4.6 i.d., 5 μm particles), 1 ml per minute of acetonitrile/water (77:23, by vol) flow and fluorescence detector (325 nm excitation and 395 emission).[18] The FAs used as standards were obtained from Sigma Chemical Co. (St. Louis, MO, USA): lauric (C12:0), myristic (C14:0), palmitic (C16:0), palmitoleic (16:1, ω9), stearic (C18:0), oleic (C18:1, ω9), linoleic (C18:2, ω6), γ-linolenic (C18:3, ω3), arachidonic (C20:4, ω6), eicosapentaenoic (C20:5, ω3) and docosahexaenoic (C22:6, ω3) acids.

Determination of the antioxidant enzyme activities

The effect of the FAs upon activities of the main antioxidant enzymes was assayed. Catalase (E.C. 1.11.1.6) activity was determined by measuring the decomposition of hydrogen peroxide at 230 nm.[20] GPx (E.C.1.11.1.9) activity was determined as described by Wendel (1981)[21] and Mannervik,[22] following the rates of NADPH oxidation at 340 nm, and 37°C, in an assay medium containing 50 mM phosphate buffer (pH 7.0), 3.6 mM sodium azide, 0.3 mM NADPH, glutathione reductase (0.25 U ml^{-1}) and 5 mM reduced glutathione. The reaction was initiated by the addition of *t*-butyl hydroperoxide (1.5 mM). One unit of GPx is defined as the amount of protein that oxidizes 1 μmol of NADPH per min. CuZn-SOD and Mn-SOD (E.C. 1.15.1.1) activities were determined according to the method of Flohé and Ötting[23] by measuring, at 25°C, the decrease in the rate of cytochrome *c* reduction in a xanthine–xanthine oxidase superoxide generating system consisting of 10 μM cytochrome c, 100 μM xanthine, 50 mM sodium phosphate buffer (pH 10) and the necessary quantity of xanthine oxidase to yield a variation of absorbance of 0.025 per min at 550 nm. One unit of SOD activity is defined as the amount of protein necessary to inhibit 50% of the rate of cytochrome *c* reduction. CuZn-SOD was differentiated from Mn-SOD by the addition of 1 mM KCN to suppress the activity of the latter. The enzyme activities were determined in a Pharmacia Biotech (Ultrospec 3000 model; Little Chalfont, UK) spectrophotometer and are expressed as UI per mg protein.

Western blotting analysis for catalase and CuZn-SOD

The Western blotting analysis was performed as described by Sambrook and collaborators.[24] Briefly, cells were lysed with the following solution: 0.1% SDS, 1% Igepal CA-630, 1% sodium deoxycholate, 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 $\mu g\,ml^{-1}$ aprotinin, 1 $\mu g\,ml^{-1}$ leupeptin, 100 $\mu g\,ml^{-1}$ phenylmethylsulfonyl fluoride and 0.5 mM EDTA. After

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centrifugation for 10 min, at 12 000*g* and 4°C, the protein concentration was determined. Forty to sixty micrograms of proteins were mixed in a buffer (3×: 100 mM Tris-HCl, pH 6.8, 5% 2-mercaptoethanol (v/v), 2% SDS, 20% glycerol, 0.01% bromophenol blue, respectively) boiled for 5 min and resolved in a 15% denaturing polyacrylamide gel (10% polyacrylamide, 380 mM Tris-HCl, pH 8.8, 0.1% SDS, 0.1% ammonium persulfate and 0.077% *N*,*N*,*N*′,*N*′-tetramethylethylenediamine). Resolved proteins were transferred to nitrocellulose membranes by electrophoresis. After 30 min incubation with blocking solution (5% low-fat milk in TBS—20 mM Tris-HCl, pH 7.5, 0.9% NaCl), the membranes were washed twice with TBST (TBS containing 0.05% Tween 20) and incubated for 2 h with rabbit anti-catalase polyclonal antibody, or with rabbit anti-CuZn-SOD polyclonal antibody, diluted 1:500 in TBST. After washing, the membranes were incubated with anti-rabbit IgG for 1 h and washed again. Protein-antibody complexes were revealed by a chemiluminescence (ECL kit) and registered on X-ray films.

Northern blotting

The total RNA was prepared using TRIZOL reagent according to the manufacturer's protocol, and 20 μg of total RNA were used for the Northern blotting analysis as previously described.[24] Briefly, oligonucleotide probes for rat catalase were labelled with [α-^{32}P] dCTP using the Readprime kit (Amersham Biosciences, Uppsala, Suécia). After electrophoresis and transferring RNA to a nylon membrane, hybridizations with labelled catalase cDNA probes were performed at 43°C for 24 h, and the membranes were washed in SSPE 2× and 0.5% SDS at 43°C for 40 min, and in SSPE 1× and 0.25% SDS at 48°C for 40 min. The membranes were exposed to Kodak film with intensifying screens at −80°C and quantified by an image analyser (Storm 840/ ImageQuaNT^{MT}—Molecular Dynamics). The loading amount of RNA was confirmed by the amount of β-actin mRNA detected by the method above.

[2-^{14}C]-thymidine incorporation (cell proliferation)

Rates of [2-^{14}C]-thymidine incorporation into DNA were evaluated in the presence and absence of FAs. Cells were seeded in 96 well plates (2.5×10^5 cells/well). After 6 h of exposure to FAs, 20 μl of a buffer solution containing [2-^{14}C] (0.02 μCi) and cold thymidine (0.2 μg) were added to each well. Cells were cultured for an additional 18 h and then harvested (Skatron Combi Multiple Cell Harvester, Suffolk, UK) on filter papers. Scintillation fluid was added to the filters and the radioactivity incorporated was determined in a Beckman Scintillation Counter (LS 6000, Meriden, CT, USA).

Protein determination

The total protein content from control and treated cells was measured by the method of Bradford,[25] using bovine albumin as standard.

Statistical analysis

Results are expressed as means ± *SD*. Statistical analyses were carried out by one-way ANOVA followed by Dunnett's test for multiple comparisons using the Prism Analysis Program (Graphpad, San Diego, CA, USA).

RESULTS

Fatty acid composition of the cell lines

In order to establish a possible relationship between the abundance of a specific FA and its toxic effect, the percentage composition of FA was determined in the Jurkat and Raji cells and in the FCS (Table 1). Raji cells contained a higher proportion of saturated FA (50%) compared to Jurkat cells (37%); this percentage was still higher in FCS (56%). On the other hand,

Table 1. Fatty acid composition of foetal calf serum and Jurkat and Raji cells

Fatty acids	Jurkat	Raji	FCS
Lauric (C 12:0)	0.4 ± 0.2	3.6 ± 0.6	2.3 ± 0.0
Miristic (C 14:0)	1.2 ± 0.1	5.4 ± 0.3	4.4 ± 0.2
Palmitic (C 16:0)	16 ± 0.3	26 ± 0.9	25 ± 0.4
Palmitoleic (C 16:1)	5.5 ± 0.3	10 ± 1.0	6 ± 0.2
Stearic (C 18:0)	13 ± 0.4	14 ± 0.7	24 ± 1.0
Oleic (C 18:1)	31 ± 0.8	30 ± 1.0	19 ± 0.6
Linoleic (C 18:2)	6 ± 0.2	3 ± 1.0	8 ± 0.1
γ-Linolenic (C 18:3)	0.2 ± 0.0	0.4 ± 0.2	0.8 ± 0.1
Arachidonic (C 20:4)	9 ± 0.2	2 ± 0.3	3 ± 0.2
Eicosapentaenoic (C 20:5)	0.8 ± 0.0	0.9 ± 0.2	1.4 ± 0.0
Docosahexaenoic (C 22:6)	13 ± 0.8	2.5 ± 0.5	4.5 ± 0.2
% of saturated FA	32	50	56
% of monounsaturated FA	36	40	26
% of PUFA ω-3	14	4	5
% of PUFA ω-6	16	6	12

The fatty acid composition (in percentage of the total) was determined by high performance liquid chromatography (HPLC) from 10^6 cells per sample. The values are presented as mean ± *SD* of three samples for FCS and four for the cell lines.
FA, fatty acids; PUFA, polyunsaturated fatty acids; FCS, fetal calf serum.

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Jurkat cells showed about three times more PUFA (30%) than Raji cells (10%). The proportion of ω-3 and ω-6 FA was almost the same in both cell lines, whereas FCS showed 17% of PUFA; 70% being of ω-6 family. The proportion of monounsaturated FAs was similar for both Jurkat and Raji cells and low in FCS. Oleic acid (OA) was the most abundant FA in both cell lines (about 30%). Of the saturated FAs, palmitic acid (PA) was the most abundant in both cell lines and also in serum. AA was the main ω-6 PUFA found in Jurkat cells (9.5%), whereas in Raji cells and in serum the most abundant ω-6 FA was linoleic acid (LA; 3 and 8%, respectively). The most abundant ω-3 PUFA was DHA, however, its proportion was different for Jurkat (13%) and Raji (2.5%) cells and in FCS (4%).

Enzyme activities

For these experiments, the FAs were added to the medium at doses below toxic concentrations. Jurkat and Raji cells behaved differently after incubation with FA. The cell lines also presented differences concerning the basal antioxidant enzyme activities. The B lymphocyte cell line (Raji cell) seems to be more protected against pro-oxidant agents, as shown by higher activities of catalase (75% higher) and total SOD (40% higher) compared to Jurkat cells—a human T lymphocyte cell line. In response to OA, Raji cells were more resistant to the decrease in catalase activity; 38% compared to 64% in Jurkat cells (Tables 2 and 3).

Catalase activity

Catalase activity was 30% higher in Raji cells (17.3 ± 1.2 IU mg^{-1} of protein) compared to Jurkat cells (9.8 ± 0.7 IU mg^{-1} of protein) under control conditions (Tables 2 and 3). The incubation of Jurkat cells with all the FAs caused a significant decrease of catalase activity. Palmitic (4.3 ± 0.7 IU mg^{-1} of protein), oleic (3.6 ± 0.5 IU mg^{-1} of protein) and eicosapentaenoic (4.3 ± 0.7 IU mg^{-1} of protein) acids reduced the catalase activity

Table 2. Antioxidant enzyme activities in Jurkat cells treated with fatty acids

		Superoxide dismutase activities		
Treatment	Catalase activity	Total	Mn-SOD	Cu/Zn-SOD
Untreated	9.7 ± 0.6	11 ± 2.0	3 ± 0.8	8.7 ± 3.0
PA 200 μM	$4.3 \pm 0.4^{**}$	$17 \pm 1.0^{**}$	4.6 ± 1.0	12.7 ± 3.0
SA 200 μM	$8 \pm 0.2^{**}$	14.5 ± 2.0	4 ± 2.0	10 ± 3.0
OA 200 μM	$3.5 \pm 0.4^{**}$	8.8 ± 1.0	2 ± 1.0	6.6 ± 1.0
LA 100 μM	$5 \pm 0.9^{**}$	13.6 ± 2.3	3 ± 1.0	9 ± 1.0
LNA 50 μM	$7.3 \pm 1.0^{**}$	14 ± 3.5	3.7 ± 1.0	10 ± 2.8
EPA 100 μM	$4.2 \pm 0.6^{**}$	14 ± 0.7	4.7 ± 0.7	9 ± 1.6

Cells were treated for 24 h with FA. After that, the antioxidant enzyme activities were determined. The values (IU mg^{-1} of protein) are expressed as means $\pm$ *SD* from eight determinations. $^{**}p < 0.01$ compared with control (ANOVA plus Dunnett).
PA, palmtic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid; LNA, γ-linolenic acid; EPA, eicosapentaenoic acid; Mn-SOD, Mn-superoxide dismutase; Cu/Zn-SOD, Cu/Zn superoxide dismutase.

Table 3. Antioxidant enzyme activities in Raji cells treated with fatty acids

		Superoxide dismutase activities		
Treatment	Catalase activity	Total	Mn-SOD	Cu/Zn-SOD
Untreated	17 ± 2.0	15.6 ± 3.0	5 ± 1.0	10 ± 2.0
PA 200 μM	$13.9 \pm 3.0^{**}$	13.5 ± 3.0	5 ± 1.0	8.5 ± 2.0
SA 200 μM	$11 \pm 1.0^{**}$	8.6 ± 0.8	3 ± 0.6	6.6 ± 0.9
OA 150 μM	$10.6 \pm 1.0^{**}$	$6 \pm 0.7^{**}$	$2 \pm 0.2^{**}$	$4 \pm 0.4^{**}$
LA 100 μM	$13.7 \pm 0.8^{*}$	10.6 ± 1.0	4.6 ± 0.7	$5.9 \pm 1.0^{*}$
LNA 50 μM	15 ± 1.0	16.9 ± 6.0	6 ± 2.0	10.8 ± 3.0
EPA 100 μM	$13.9 \pm 2.0^{**}$	11 ± 2.0	3.9 ± 1.0	7 ± 1.0

Cells were treated for 24 h with different FA. After that, the antioxidant enzyme activities were determined. The values (IU mg^{-1} of protein) are expressed as means $\pm$ *SD* ($n = 8$). $^{*}p < 0.05$; $^{**}p < 0.01$ (ANOVA plus Dunnett).
PA, palmtic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid; LNA, γ-linolenic acid; EPA, eicosapentaenoic acid; Mn-SOD, Mn-superoxide dismutase; Cu/Zn-SOD, Cu/Zn-superoxide dismutase.

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by at least 50% in Jurkat cells compared to control (Table 2). LA was intermediate in its effect on reducing catalase activity (50%), whereas stearic and γ-linolenic acids (LNAs) were less effective (reductions of 18 and 25%, respectively). In Raji cells, catalase activity (17 ± 2 IU mg^{-1} of protein) was significantly reduced after incubation with stearic (11.3 ± 1.2 IU mg^{-1} of protein) and oleic (10.7 ± 1.2 IU mg^{-1} of protein) acids only. Palmitic, linoleic and eicosapentaenoic acids caused a 25% reduction of catalase activity in Raji cells (Table 3). This effect was less pronounced than that observed in Jurkat cells (Table 2).

Superoxide dismutase activity

The FAs did not induce significant changes in activities of either Mn- or CuZn-SOD in either cell line (Tables 2 and 3). In Jurkat cells, only PA induced an increase of 56% in total SOD activity (11 ± 3 vs. 17 ± 1). In Raji cells, OA reduced total SOD activity by 40% compared to the control (6 ± 0.7 vs.15 ± 3). This decrease in total SOD activity was due to a reduction of both Mn- and CuZn-SOD activities. LA was able to reduce the activity of CuZn-SOD (6 ± 1 vs. 10 ± 2) in Raji cells only but it did not affect total SOD activity. The activity of GPx was not detected in either cell line by the method used.

Western and Northern blotting analysis of catalase and Mn- and CuZn-superoxide dismutases

FAs did not affect the protein content of catalase or of both SODs (data not shown). The same result was observed for catalase gene expression, as determined by Northern blotting analysis (data not shown).

Effect of **α***-tocopherol treatment*

To address the possible involvement of lipid peroxidation in the effects observed, the cells were pre-incubated, for 30 min, with α-tocopherol (40 μM). After that, cells were treated with FA at toxic concentrations and analysed for cell viability. The pre-incubation with α-tocopherol lowered the toxicity of LNA on Jurkat cells (Figure 1). This protective effect of α-tocopherol was less pronounced with the increase in LNA concentration in Jurkat cells. The viability of the cells was of 80%, 50% and 25% at 100, 150 and 200 μM LNA, respectively, after α-tocopherol treatment, compared with α-tocopherol

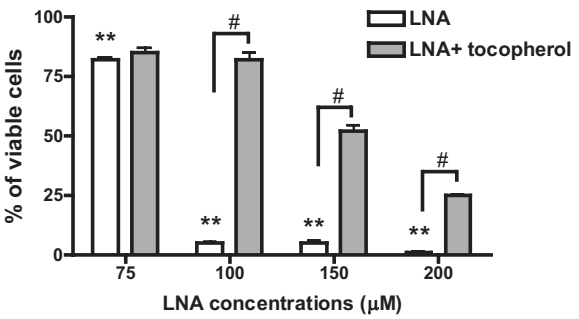

Figure 1. Effect of α-tocopherol on γ-linolenic acid toxicity in Jurkat cells: cells were previously treated with 40 μM α-tocopherol for 30 min and afterwards cultured for 24 h in the presence of LNA at different concentrations before cell viability was analysed. The values expressed in percentages are presented as means ± *SD* from six samples. $^{**}p < 0.01$ for comparison with untreated cells (98 ± 2; ANOVA plus Dunnett); $^{\#}p < 0.01$ for comparison with cell cultured in the absence of tocopherol (Unpaired Student *t*-test). LNA, γ-linolenic acid

untreated cells (1–5% of viable cells). Raji cells treated with OA, LA, LNA and EPA at 200 μM showed a very low viability (approximately 1%). After α-tocopherol treatment the percentage of viable Raji cells was raised to 90% for OA, 75% for LA, 90% for LNA and 80% for EPA (Figure 2).

[2-^{14}C]-thymidine incorporation

[2-^{14}C]-thymidine incorporation assay is indicative of cell proliferation. In Jurkat cells, palmitic and stearic

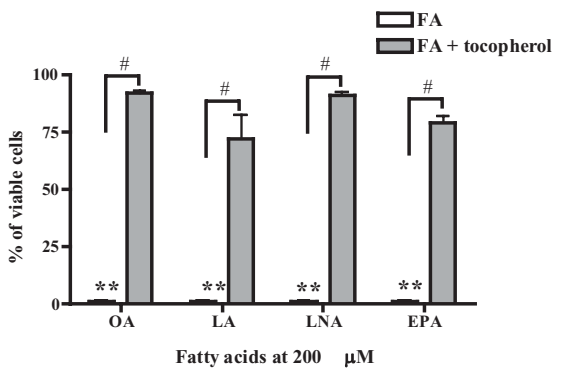

Figure 2. Effect of α-tocopherol on FA toxicity in Raji cells: cells were previously treated with 40 μM tocopherol for 30 min and afterwards cultured for 24 h in the presence of FA at different concentrations before cell viability was analysed. The values expressed in percentages are presented as means ± *SD* from six samples. $^{**}p < 0.01$ for comparison with untreated cells (98 ± 2; ANOVA plus Dunnett); $^{\#}p < 0.01$ for comparison with cell cultured in the absence of tocopherol (Unpaired Student's *t*-test). FA, fatty acids; OA, oleic acid; LA, linoleic acid; LNA, γ-linolenic acid; EPA, eicosapentaenoic acid

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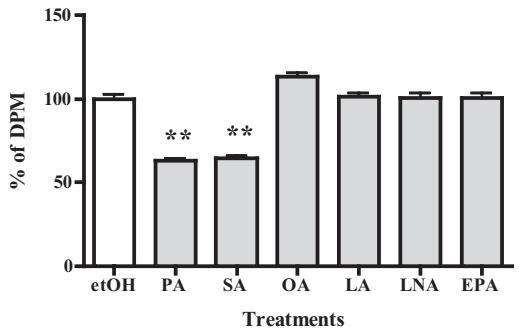

Figure 3. [2-^{14}C]-thymidine incorporation into Jurkat cells: cells were cultured in RPMI 1640 medium supplemented with FCS at 10% in 96 well plates for 24 h. Cells were incubated with isolated FAs or ethanol (control condition). Thymidine solution (cold thymidine 0.2 μg and [2-^{14}C]-thymidine 0.02 μCi per well) was added after 6 h culture with FAs. After 18 h culture, cells were collected and radioactivity determined. etOH, ethanol; PA, palmitic acid (0.2 mM); SA, stearic acid (0.2 mM); OA, oleic acid (0.2 mM); LA, linoleic acid (0.1 mM); LNA, γ-linolenic acid (0.05 mM); EPA, eicosapentaenoic acid (0.1 mM). Values expressed as percentages of DPM variation are presented as mean ± *SD* from 10 determinations compared with control (etOH = 568 ± 55). $^{*}p < 0.05$ and $^{**}p < 0.01$ for comparison with control (ANOVA plus Dunnett)

acids decreased thymidine incorporation by 35% compared to the control (Figure 3). The remaining FA did not affect Jurkat cell proliferation. In Raji cells, the FA effect was somehow different (Figure 4). PA caused a less pronounced effect on cell proliferation (a decrease of 15%) compared with its effect on Jurkat cells. On the other hand, LNA reduced Raji cell proliferation by 50%. Different from the results observed for Jurkat cells, EPA increased Raji cell proliferation by 30%.

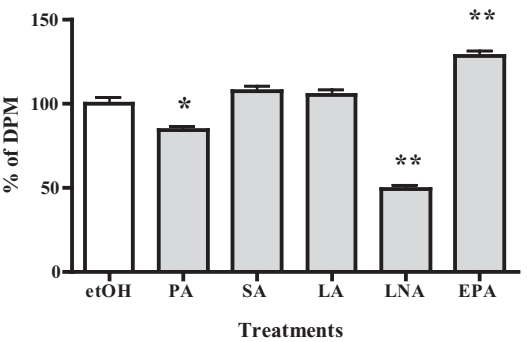

Figure 4. [2-^{14}C]-thymidine incorporation into Raji cells: cells were cultured in RPMI 1640 medium supplemented with FCS at 10% in 96 well plates for 24 h. Cells were incubated with isolated FAs or ethanol (control condition). Thymidine solution (cold thymidine 0.2 mg and [2-^{14}C]-thymidine 0.02 μCi per well) was added after 6 h culture with FAs. After 18 h culture, cells were collected and radioactivity determined. etOH, ethanol; PA, palmitic acid (0.2 mM); SA, stearic acid (0.2 mM); LA, linoleic acid (0.1 mM); LNA, γ-linolenic acid (0.05 mM); EPA, eicosapentaenoic acid (0.1 mM). Values expressed as percentages of DPM variation are presented as mean ± *SD* from 10 determinations compared with control (etOH = 323 ± 39). $^{*}p < 0.05$ and $^{**}p < 0.01$ for comparison with control (ANOVA plus Dunnett)

DISCUSSION

Antioxidant enzymes are regulated by several factors. Oxidative status of the cell is the primary regulator of expression and activity of these enzymes.[26,27] The activities of antioxidant enzymes are also changed by cell differentiation, aging, inflammation and high levels of melatonin, TNF-α, IL-1β [28–30] and prolactin.[31] N-nitro-L-arginine methyl ester hydrochloride, an inhibitor of nitric oxide synthase, increases catalase activity.[32]

The balance between ROS formation and degradation can be disturbed not only by an increase in its production but also by a decrease in the antioxidant enzyme activities. In phagocytes, AA stimulates superoxide generation, being cytotoxic at physiological and supraphysiological concentrations.[33,34] In addition to stimulating ROS production,[35] the present study provides evidence that FA also decreases the activitiy of catalase in Jurkat and Raji cells. FAs, mainly the PUFAs ones, are also putative targets for free radical propagation, during which lipid peroxides are generated, being deleterious for the tissues.[36–38]

Treating glial cells with AA, EPA and DHA, Leonardi *et al*.[36] observed a more pronounced effect of DHA to increase catalase activity and to decrease glutathione content. These results are in contrast to our findings with leukaemia cell lines. The lowering effects of the FA on catalase activity in Jurkat and Raji cells did not follow any correlation with the carbon chain length and the unsaturation degree as also found by Hawkins *et al*.[39] studying FA cytotoxicity. Interestingly, PA increased SOD activity in Jurkat cells, whereas oleic decreased this enzyme activity in Raji cells.

The relationship between the abundance of the FA and its effect on catalase activity was also investigated. OA was the most abundant FA in both cell lines and also the most effective one in decreasing catalase activity. On the other hand, the less abundant FA in both cell lines, LNA presented the highest toxicity for both Jurkat and Raji cells.

The effects of the FA to reduce catalase activity was more pronounced in Jurkat cells than in Raji cells. However, the latter cell line was more susceptible to the toxic effect of the FA. These findings point out that

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the effect of the FA on catalase activity and cell death varied with the cell type.

The possible accumulation of H_2O_2 derived from the decrease in catalase activity may have an important role for the reduction in lymphocyte proliferation induced by some FA. In Jurkat cells, the saturated palmitic and stearic acids were able to significantly reduce cell proliferation. On the other hand, in Raji cells, this inhibition effect was observed after incubation with PA and LNA. EPA reduced catalase activity but enhanced Raji cell proliferation. Interestingly, OA, LA, LNA and EPA decreased catalase activity but did not decrease Jurkat cell proliferation. This suggests that the FA may affect cell proliferation by other mechanisms in addition to a change in redox status.

In order to test the mechanism by which the FA reduced catalase activity, Western and Northern blotting analyses were performed. As we did not observe changes in RNA and protein levels, the FA regulation of calatase activity seems to occur at a post-translational level, as also observed by Mayo *et al*.[40] in PC12 and SK-N-SH cells. These authors observed an increase in unstable mRNA of GPx and CuZn-SOD after incubation with melatonin. In addition, acylation of proteins is a well-known mechanism to regulate protein function and should be investigated.

Lipid peroxidation has been recognized to play an important role in the toxicity of FAs.[41] Administration of vitamin E prevents lipid peroxidation and has been shown to possess beneficial effects in high oxidative stress conditions such as hypothyroidism[42] and high doses of glucocorticoid.[43] In our study, the addition of α-tocopherol prevented the cytotoxic effects of FAs, such as oleic, linoleic, LNAs and EPA on Raji cells and LNA on Jurkat cells. These findings support the supposition that FA induced oxidative stress in both cell types but mainly in Raji cells. The increase in oxidative stress is partially due to a reduction in catalase activity.[44] Increased oxidative stress associated with decreased antioxidant capacity and lowered enzyme activities were also reported in children with acute pneumonia[45] and patients with breast cancer.[46]

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REFERENCES

1. Hughes DA, Pinder AC. Influence of n-3 polyunsaturated fatty acids (PUFA) on the antigen-presenting function of human monocytes. *Biochem Soc Trans* 1996; **24**: 389S.
2. Grimble RF. Nutritional modulation of cytokine biology. *Nutrition* 1998; **14**: 634–640.
3. Kelley DS, Taylor PC, Nelson GJ, *et al*. Docosahexaenoic acid ingestion inhibits natural killer cell activity and production of inflammatory mediators in young healthy men. *Lipids* 1999; **34**: 317–324.
4. Calder PC. Polyunsaturated fatty acids, inflammation, and immunity. *Lipids* 2001; **36**: 1007–1024.
5. Thies F, Nebe-von-Caron G, Powell JR, Yaqoob P, Newsholme EA, Calder PC. Dietary supplementation with gamma-linolenic acid or fish oil decreases T lymphocyte proliferation in healthy older humans. *J Nutr* 2001; **131**: 1918–1927.
6. Peres CM, Otton R, Curi R. Modulation of lymphocyte proliferation by macrophages and macrophages loaded with arachidonic acid. *Cell Biochem Funct* 2005; **23**: 373–381.
7. Finstad HS, Drevon CA, Kulseth MA, Synstad AV, Knudsen E, Kolset SO. Cell proliferation, apoptosis and accumulation of lipid droplets in U 937-1cells incubated with eicosapentaenoic acid. *Biochem J* 1998; **336**: 451–459.
8. Mainou-Fowler T, Proctor SJ, Dickinson AM. Gamma-linolenic acid induces apoptosis in B-chronic lymphocytic leukaemia cells in vitro. *Leuk Lymphoma* 2001; **40**: 393–403.
9. Koller M, Wachtler P, David A, Muhr G, Konig W. Arachidonic acid induces DNA-fragmentation in human polymorphonuclear neutrophil granulocytes. *Inflammation* 1997; **21**: 463–474.
10. Pallardy M, Perrin-Wolff LR, Biola A. Cellular stress and apoptosis. *Toxicol Vitr* 1997; **11**: 573–578.
11. Lima TM, Kanunfre CC, Pompeia C, Verlengia R, Curi R. Ranking the toxicity of fatty acids on Jurkat and Raji cells by flow cytometric analysis. *Toxicol Vitr* 2002; **16**: 741–747.
12. Cervinka M, Puza V. Cellular and molecular stress regulation and apoptosis; apoptosis and necrosis: dynamics of structural changes in cells cultivated in vitro after treatment with xenobiotics. *Toxicol Vitr* 1995; **9**: 387–396.
13. Hardwick SJ, Carpenter KL, Law NS, *et al*. Toxicity of polyunsaturated fatty acid esters for human monocyte-macrophages: the anomalous behaviour of cholesteryl linolenate. *Free Radic Res* 1997; **26**: 351–362.
14. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957; **226**: 497–509.
15. Azevedo-Martins AK, Monteiro AP, Lima CL, Lenzen S, Curi R. Fatty acid-induced toxicity and neutral lipid accumulation in insulin-producing RINm5F cells. *Toxicol Vitr* 2006; **20**(7): 1106–1113.
16. Hamilton S, Hamilton RJ, Sewell PA. Extraction of lipids and derivative formation. In *Lipid Analysis: A Practical Approach*, Hamilton RJHS (ed.). Press at Oxford University Press: Oxford, 1992; 718–724.
17. Beyer ES, Jensen LN. Overestimation of the cholesterol content of egg. *Agric Food Chem* 1989; **37**: 917–920.
18. Nishiyama-Naruke A, de Sousa JAA, Carnelós Filho M, Curi R. HPLC determination of underivatized fatty acid saponified at low temperature analysis of fatty acids in oils and tissues. *Anal Lett* 1998; **31**: 2565–2576.
19. Abushufa R, Reed P, Weinkove C. Fatty acids in erythrocytes measured by isocratic HPLC. *Clin Chem* 1994; **40**: 1707–1712.

DOI: 10.1002/cbf

20. Beutler E. Catalase, in red cell metabolism. In *Red Cell Metabolism*, Beutler E (ed.). Grune and Stratton: New York, 1975; 88–89.
21. Wendel A. Glutathione peroxidase. *Methods Enzymol* 1981; **77**: 325–333.
22. Mannervik B. Glutathione peroxidase. *Methods Enzymol* 1985; **113**: 490–495.
23. Flohe L, Gunzler WA. Assays of glutathione peroxidase. *Methods Enzymol* 1984; **105**: 114–121.
24. Sambrook J, Fritsch E, Maniatis T. (eds). *Molecular Cloning: A Laboratory Manual* (2nd edn), Harbor. CS (ed.). Cold Spring Harbor Laboratory Press: NY, 1989.
25. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248–254.
26. Warner BB, Stuart L, Gebb S, Wispe JR. Redox regulation of manganese superoxide dismutase. *Am J Physiol* 1996; **271**: L150–L158.
27. Franco AA, Odom RS, Rando TA. Regulation of antioxidant enzyme gene expression in response to oxidative stress and during differentiation of mouse skeletal muscle. *Free Radic Biol Med* 1999; **27**: 1122–1132.
28. Jones PL, Ping D, Boss JM. Tumor necrosis factor alpha and interleukin-1beta regulate the murine manganese superoxide dismutase gene through a complex intronic enhancer involving C/EBP-beta and NF-kappaB. *Mol Cell Biol* 1997; **17**: 6970–6981.
29. Rogers RJ, Chesrown SE, Kuo S, Monnier JM, Nick HS. Cytokine-inducible enhancer with promoter activity in both the rat and human manganese-superoxide dismutase genes. *Biochem J* 2000; **347**: 233–242.
30. Tunez I, Munoz MC, Medina FJ, Salcedo M, Feijoo M, Montilla P. Comparison of melatonin, vitamin E and L-carnitine in the treatment of neuro- and hepatotoxicity induced by thioacetamide. *Cell Biochem Funct* 2007; **25**(2): 119–127.
31. Rodriguez C, Mayo JC, Sainz RM, *et al*. Regulation of antioxidant enzymes: a significant role for melatonin. *J Pineal Res* 2004; **36**: 1–9.
32. Kremser K, Stangl H, Pahan K, Singh I. Nitric oxide regulates peroxisomal enzyme activities. *Eur J Clin Chem Clin Biochem* 1995; **33**: 763–774.
33. Pompeia C, Lima T, Curi R. Arachidonic acid cytotoxicity: can arachidonic acid be a physiological mediator of cell death? *Cell Biochem Funct* 2003; **21**: 97–104.
34. Pompeia C, Freitas JJ, Kim JS, Zyngier SB, Curi R. Arachidonic acid cytotoxicity in leukocytes: implications of oxidative stress and eicosanoid synthesis. *Biol Cell* 2002; **94**: 251–265.
35. Pompeia C, Cury-Boaventura MF, Curi R. Arachidonic acid triggers an oxidative burst in leukocytes. *Braz J Med Biol Res* 2003; **36**: 1549–1560.
36. Leonardi F, Attorri L, Di Benedetto R, *et al*. Effect of arachidonic, eicosapentaenoic and docosahexaenoic acids on the oxidative status of C6 glioma cells. *Free Radic Res* 2005; **39**: 865–874.
37. Lopes LR, Laurindo FRM, Mancini-Filho J, Curi R, Sannomiya P. NADPH-oxidase activity and lipid peroxidation in neutrophils from rats fed fat-rich diets. *Cell Biochem Funct* 1999; **17**: 57–64.
38. Silva LP, Lemos APC, Cuve R, Azevedo RB. Effects of fish oil treatment on bleomycin-induced pulmonary fibrosis in mice. *Cell Biochem Funct* 2006; **24**: 387–396.
39. Hawkins RA, Sangster K, Arends MJ. Apoptotic death of pancreatic cancer cells induced by polyunsaturated fatty acids varies with double bond number and involves an oxidative mechanism. *J Pathol* 1998; **185**: 61–70.
40. Mayo JC, Sainz RM, Antoli I, Herrera F, Martin V, Rodriguez C. Melatonin regulation of antioxidant enzyme gene expression. *Cell Mol Life Sci* 2002; **59**: 1706–1713.
41. Ding WQ, Vaught JL, Yamauchi H, Lind SE. Differential sensitivity of cancer cells to docosahexaenoic acid-induced cytotoxicity: the potential importance of down-regulation of superoxide dismutase 1 expression. *Mol Cancer Ther* 2004; **3**: 1109–1117.
42. Sarandol E, Tas S, Dirican M, Serdar Z. Oxidative stress and serum paraoxonase activity in experimental hypothyroidism: effect of vitamin E supplementation. *Cell Biochem Funct* 2005; **23**: 1–8.
43. Beytut E, Erisir M, Aksakal M. Effects of additional vitamin E and selenium supply on antioxidative defence mechanisms in the kidney of rats treated with high doses of glucocorticoid. *Cell Biochem Funct* 2004; **22**: 59–65.
44. Wei YH, Lee HC. Oxidative stress, mitochondrial DNA mutation, and impairment of antioxidant enzymes in aging. *Exp Biol Med (Maywood)* 2002; **227**: 671–682.
45. Cemek M, Caksen H, Bayiroglu F, Cemek F, Dede S. Oxidative stress and enzymic-non-enzymic antioxidant responses in children with acute pneumonia. *Cell Biochem Funct* 2006; **24**: 269–273.
46. Erten Sener D, Gonenc A, Akinci M, Torun M. Lipid peroxidation and total antioxidant status in patients with breast cancer. *Cell Biochem Funct* 2006; (epub ahead of print).

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