PROSTAGLANDINS LEUKOTRIENES AND ESSENTIAL FATTY ACIDS

Prostaglandins Leukotrienes and Essential Fatty Acids (1994) 51, 33–40

Suppression of Human T-Cell Growth In Vitro by Cis-unsaturated Fatty Acids: Relationship to Free Radicals and Lipid Peroxidation

N. Madhavi, U. N. Das, P. Surya Prabha, G. Sravan Kumar, R. Koratkar and P. Sangeeta Sagar

Department of Medicine, The Nizam's Institute of Medical Sciences, Punjagutta, Hyderabad-500482, India (Reprint requests to UND)

ABSTRACT. **Cis-unsaturated fatty acids such as dihomogamma-linolenic acid (DGLA), arachidonic acid (AA) and eicosapentaenoic acid (EPA), which form precursors to 1, 2 and 3 series prostaglandins (PGs), have been shown to suppress human T-cell growth in vitro by a prostaglandin E (PGE)-independent mechanism. In an earlier study, we showed that these fatty acids can induce free radical generation in human neutrophils and tumor cells. Here we show that cis-unsaturated fatty acids augment free radical generation and lipid peroxidation in human T-cells. The growth suppressive action of cis-unsaturated fatty acids on human T-cells could be blocked by anti-oxidant, vitamin E and the superoxide anion quencher superoxide dismutase. These results suggest that c-UFAs-induced cell growth suppression is a free radical dependent process.**

INTRODUCTION

Prostaglandins (PGs), thromboxanes (TXs) and leukotrienes (LTs), known as eicosanoids, are derived from polyunsaturated fatty acids (PUFAs). There are two types of PUFAs: the omega 6 (n-6) group is derived from linoleic acid (LA) and the omega 3 (n-3) group from alpha-linolenic acid (ALA). Both LA and ALA are believed to be desaturated and elongated by the same set of enzymes (1). Dihomogamma-linolenic acid (DGLA) and arachidonic acid (AA) derived from LA, and eicosapentaenoic acid (EPA) derived from ALA form precursors to 1, 2 and 3 series PGs.

Diets enriched with DGLA and EPA have been shown to reduce inflammation and tissue injury in animal models (2). Pain and inflammation in patients with rheumatoid arthritis and psoriasis can be reduced by dietary supplementation with fish oil rich in EPA and plant seed oil rich in gamma-linolenic acid (GLA) (3, 4). It has also been shown that dietary supplementation with fish oil can decrease the synthesis of interleukin 1 (IL-1) and tumor necrosis factor, which are known to have pro-inflammatory actions (5). In an elegant study Santoli et al (6) have demonstrated that IL-2 dependent human T-cell growth can be suppressed by DGLA, AA, and EPA and that this inhibitory action is independent of PGE. Our earlier studies (7, 8) and those of others (9–12) have shown that PUFAs have a selective tumoricidal action and that this action cannot be blocked by cyclooxygenase and lipoxygenase inhibitors. We have also demonstrated that these fatty acids augment free radical generation both in tumor cells and normal human leukocytes (13, 14). In this report, we demonstrate that:

1. PUFAs suppress human T-cell proliferation.
2. The inhibitory activity of the fatty acids can be blocked by vitamin E and superoxide dismutase.
3. That the fatty acids can augment free radical generation and lipid peroxidation in lymphocytes. These findings indicate that PUFAs-induced suppression of T-cell proliferation is a free radical dependent process.

Date received 28 June 1993
Date accepted 20 December 1993

MATERIALS AND METHODS

Isolation of lymphocytes

Peripheral blood lymphocytes from healthy donors were separated on a Ficoll-Hypaque gradient. The separated lymphocytes were grown at 37°C in RPMI 1640 medium supplemented with 10% human AB serum, glutamine and anti-biotics. T-lymphocytes were stimulated to grow by phytohaemagglutinin (PHA). It was observed that less than 1 % of monocytes/macrophages are present in the lymphocytes separated.

The effect of fatty acids on the proliferation of T-cells was determined by measuring [3H] thymidine incorporation in cells seeded at 1 × 10–5 cells/well in 24-well culture plates in the presence of PHA. After 24, 48 and 72 h

incubation, 1 uCi of [3H] thymidine was added to each well and incorporation measured 24 h later. All data are presented as mean counts per min from triplicate cultures.

The stock solutions of fatty acids were prepared in distilled ethanol and stored under nitrogen. Just prior to use, fatty acids were taken from the stock solutions and diluted in sterile saline such that the final ethanol concentration was not more than 0.1% for any given concentration of fatty acid used. The control cultures received 0.1% of ethanol. Vitamin E (alpha-tocopherol obtained from Sigma) was dissolved in ethanol and just before use it was further diluted in sterile saline.

Estimation of free radicals and lipid peroxides

Superoxide anion, hydrogen peroxide, and lipid peroxidation products formed and released by lymphocytes with and without PUFA treatments were measured by nitroblue tetrazolium, horse radish peroxidase, and thiobarbi-turic acid methods as described earlier (13–15). All chemicals used in this study were obtained from Sigma Chemical Company, USA. [3H] thymidine (specific activity 16 000 mCi/m mole) was purchased from BARC, Bombay, India. Data obtained were analyzed using Students t-test and Scheffe's test.

RESULTS

All the fatty acids tested were found to produce a dose dependent inhibition of T-cell proliferation as measured by [3H] TdR incorporation (Fig. 1). Of all the fatty acids tested, AA, ALA and DGLA were found to be less inhibitory compared to other fatty acids. Since the studies performed by Santoli et al (6) and our own studies (Fig. 2) have shown that human T-cell growth inhibition by fatty acids is not dependent on cyclooxygenase and lipoxygenase pathways, we studied the possible involvement of free radicals in this process. The results presented in Figures 3 and 4 suggest that all the fatty acids tested especially LA, GLA, EPA and docosahexaenoic acid (DHA) can indeed augment both superoxide and H_2O_2 production in the T-cells. Similar to their weak inhibitory action on T-cell proliferation, ALA, AA and DGLA were found to be less potent stimulators of free radical generation compared to other fatty acids. This indicated that both free radical generation and the inhibitory action of fatty acids on T-cell proliferation are closely related. Similar to free radical generation, the fatty acids were also found to augment lipid peroxidation in the T-cells as measured by thiobarbituric acid method (Fig. 5).

To verify whether free radicals are indeed the mediators of fatty acid induced inhibition of T-cell proliferation, we tested the effect of various anti-oxidants and free radical quenchers on T-cell growth. Results shown in Figure 2 clearly indicate that LA, GLA and DHA induced inhibition of T-cell proliferation can be completely reversed by vitamin E, an anti-oxidant, and superoxide dismutase, a superoxide anion quencher whereas both mannitol and catalase could do so only partially, suggesting that H_2O_2 and hydroxyl radicals do not have a major role in the growth inhibitory action of fatty acids on T-cell suppression. Indomethacin and Nordihydroguaretic acid (NDGA) which are inhibitors of cyclooxygenase and lipoxygense pathways respectively have no effect on T-cell proliferation, indicating that as observed by Santoli et al (6) both PGs and LTs do not have a role in the growth inhibitory action of fatty acids.

DISCUSSION

Human T-lymphocytes can be activated by various stimuli such as antigens, lectins, and antibodies directed to cell surface membrane structures. Activated T-cells are believed to have a central role in the pathogenesis of many autoimmune and inflammatory conditions. Here we have demonstrated that essential fatty acids (LA and ALA) and their metabolites (GLA, DGLA, AA, EPA and DHA) can inhibit lectin-stimulated T-cell proliferation. Our results are similar to those of Santoli et al (6) who have shown that IL-2 dependent T-cell growth in vitro can be suppressed by DGLA, AA and EPA. Their studies (6) have demonstrated that little or no PG was produced by fatty acid treated T-cells, and indomethacin, a PG synthase inhibitor, could not reverse the anti-proliferative effects of the fatty acids. In their IL-2 propagated T-cell cultures, high affinity binding to 125I-labelled IL-2 was not affected by either preincubation with or addition of PGE and fatty acids. Thus, they could not identify the precise mechanism by which these agents suppress the in vitro growth of IL-2 dependent T-cells.

In our earlier studies, we and others have shown that essential fatty acids and their metabolites can suppress the growth of malignant cells and at appropriate concentrations can induce tumor cell death (7, 12). This fatty acid induced tumoricidal action was observed to be independent of cyclooxygenase and lipoxygenase pathways. Further studies revealed that these fatty acids can augment free radical generation in tumor cells but not in normal cells and that their tumoricidal action can be blocked by anti-oxidants and SOD (7–13). Taking clues from these studies and from those of Santoli et al (6), we studied the possible role of free radicals in fatty acid-induced T-cell growth suppression. Similar to our earlier results with tumor cells, T-cell growth inhibition by fatty acids seems to be a free radical dependent process and is independent of cyclooxygenase and lipoxygenase pathways. Overall, our data indicate that fatty acids have potent suppressive effects on T-cell proliferation and that it is a free radical dependent process. Since fatty acids have the ability to suppress IL-2 production

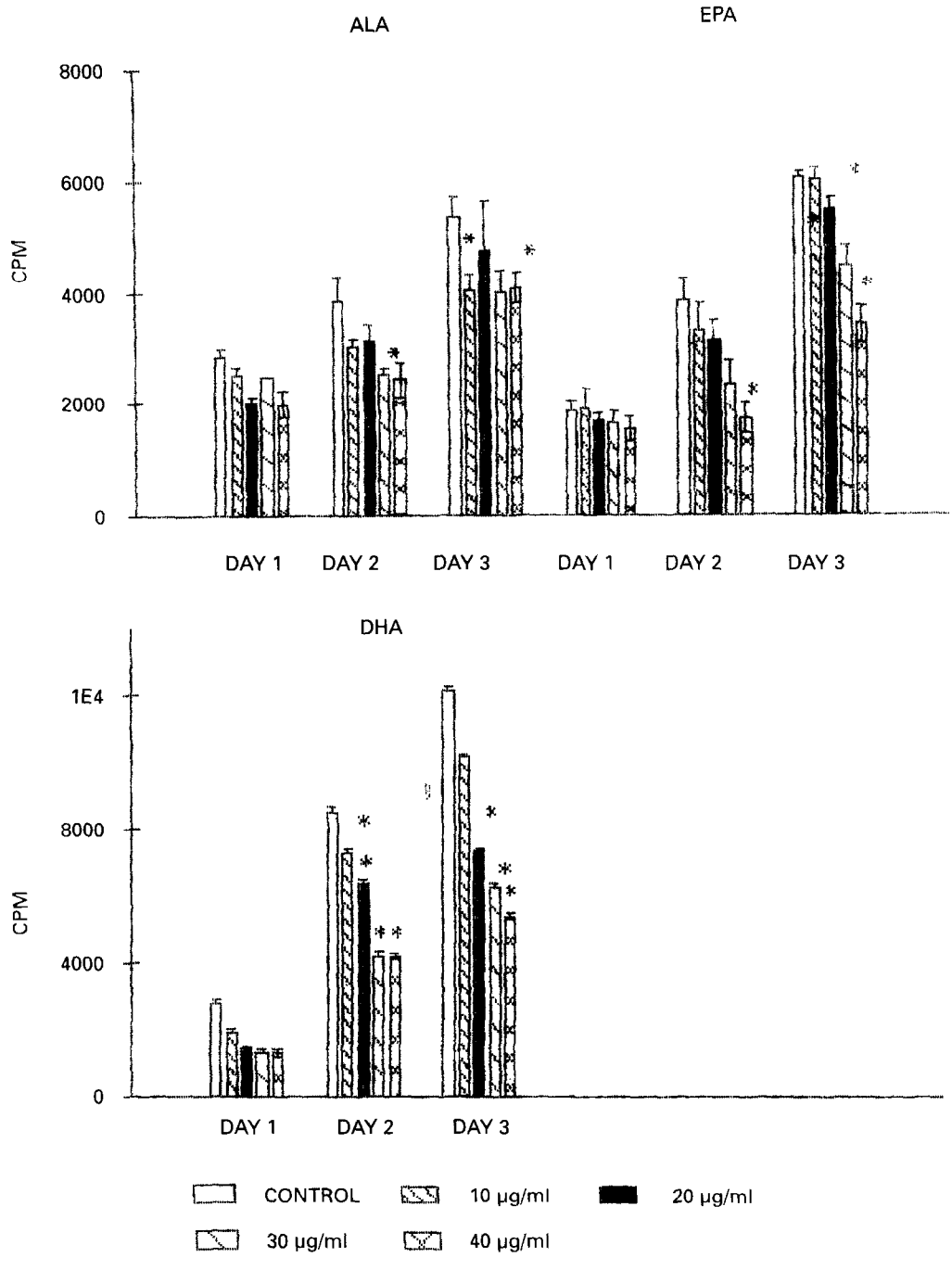

Fig. 1 Effect of fatty acids on T-cell growth. The effect of fatty acids on the proliferation of T-cells was determined by measuring [3H] thymidine incorporation. Cells were seeded at s density of 1×10^5 cells/well with 1% PHA and different fatty acids. At the end of 24, 48 and 72 h incubation, 1 uCi of [3H] thymidine was added to each well and incorporation measured 24 h later. *P ≤ 0.05 compared to control.

by stimulated human T-cells via a PGE-independent mechanism (16) and as uncontrolled T-cell proliferation contributes to overt autoimmune and inflammatory reactions, increased consumption of essential fatty acids may be considered in the treatment of these conditions.

Acknowledgements

This work was supported by a grant from Department of Science and Technology of India and Indian National Science Academy to Dr U.N. Das. NM and RK are in receipt of a UGC Junior Research Fellowship, GSK was a Junior Research Fellow of DST, PSP was a Research Associate of ICMR and PSS is a Senior Research Fellow of CSIR.

References

1. Das U N, Horrobin D F, Begin M E et al. Clinical significance of essential fatty acids. Nutrition 1988; 4: 337–341.

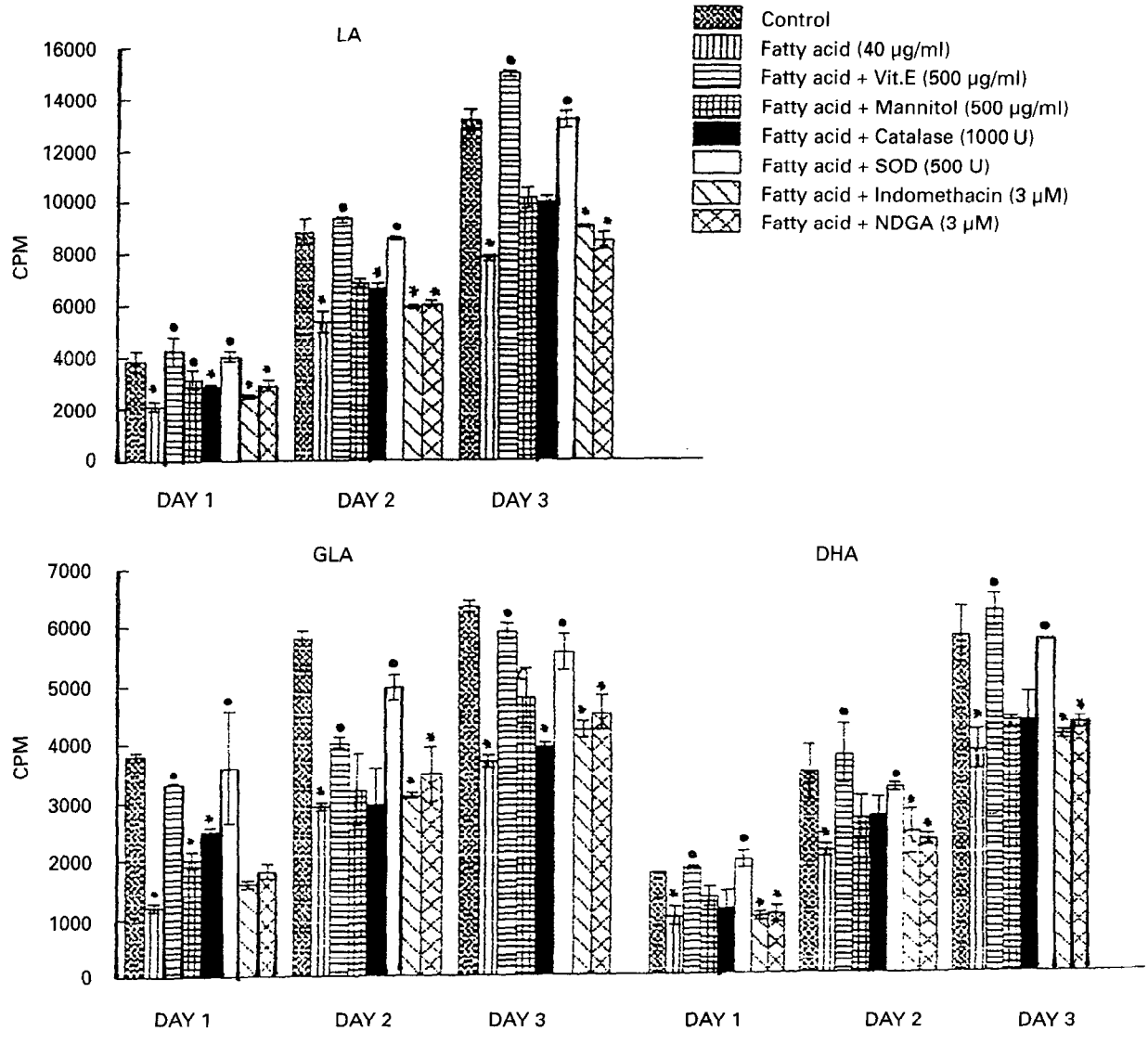

Fig. 2 Effect of anti-oxidants and inhibitors of cyclo and lipoxygenase on thymidine incorporation in fatty acid treated lymphocytes. The effect of anti-oxidants and inhibitors of cyclooxygenase and lipoxygenase on T-cell growth treated with various fatty acids was studied by measuring [3H] thymidine incorporation. 1×10^5 cells/well were seeded in 24-well culture plates with 1% PHA. Different anti-oxidants and cyclooxygenase and lipoxygenase inhibitors were added. At the end of 24, 48 and 72 h incubation 1 uCi of thymidine was added to each well and incorporation measured 24 h later. *$P < 0.05$ compared to control. *$P \leq 0.05$ compared to fatty acid control.

2. Tate G A, Mandell B F, Karmali R A et al. Suppression of monosodium urate crystal induced acute inflammation by diets enriched with gamma-linolenic acid and eicosapentaenoic acid. Arthritis Rheum 1988; 31: 1543–1551.
3. Kremer J M, Bigaonette J, Michalek A et al. Effects of manipulation of dietary fatty acids on clinical manifestations of rheumatoid arthritis. Lancet 1985; (ii): 184–187.
4. Tate G H, Mandell B F, Karmali R A et al. Suppression of monosodium urate crystal-induced acute inflammation by diets enriched with gamma-linolenic acid and eicosapentaenoic acid. Arthritis Rheum 1988; 31: 1543–1551.
5. Endres S, Chorbani R, Kelly V E et al. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mono-nuclear cells. N Engl J Med 1989; 320: 265–271.
6. Santoli D, Phillips P D, Colt T L, Zurier R B. Suppression of interleukin-2 dependent human T cell growth in vitro by Prostaglandin E (PGE) and their precursor fatty acids. J Clin Invest 1990; 85: 424–432.
7. Begin M E, Das U N, Ells G, Horrobin D F. Selective killing of tumor cells by Polyunsaturated fatty acids. Prostaglandins Leukot Med 1985; 19: 177–186.
8. Begin M E, Ells G, Das U N, Horrobin D F. Differential killing of human carcinomal cells supplemented with n-3 and n-6 Polyunsaturated fatty acids. J Natl Cancer Inst 1986; 77: 1053–1062.
9. Booyens J, Engelbrecht P, Le Roux S et al. Some effects of the essential fatty acids linoleic acid and alpha-linolenic

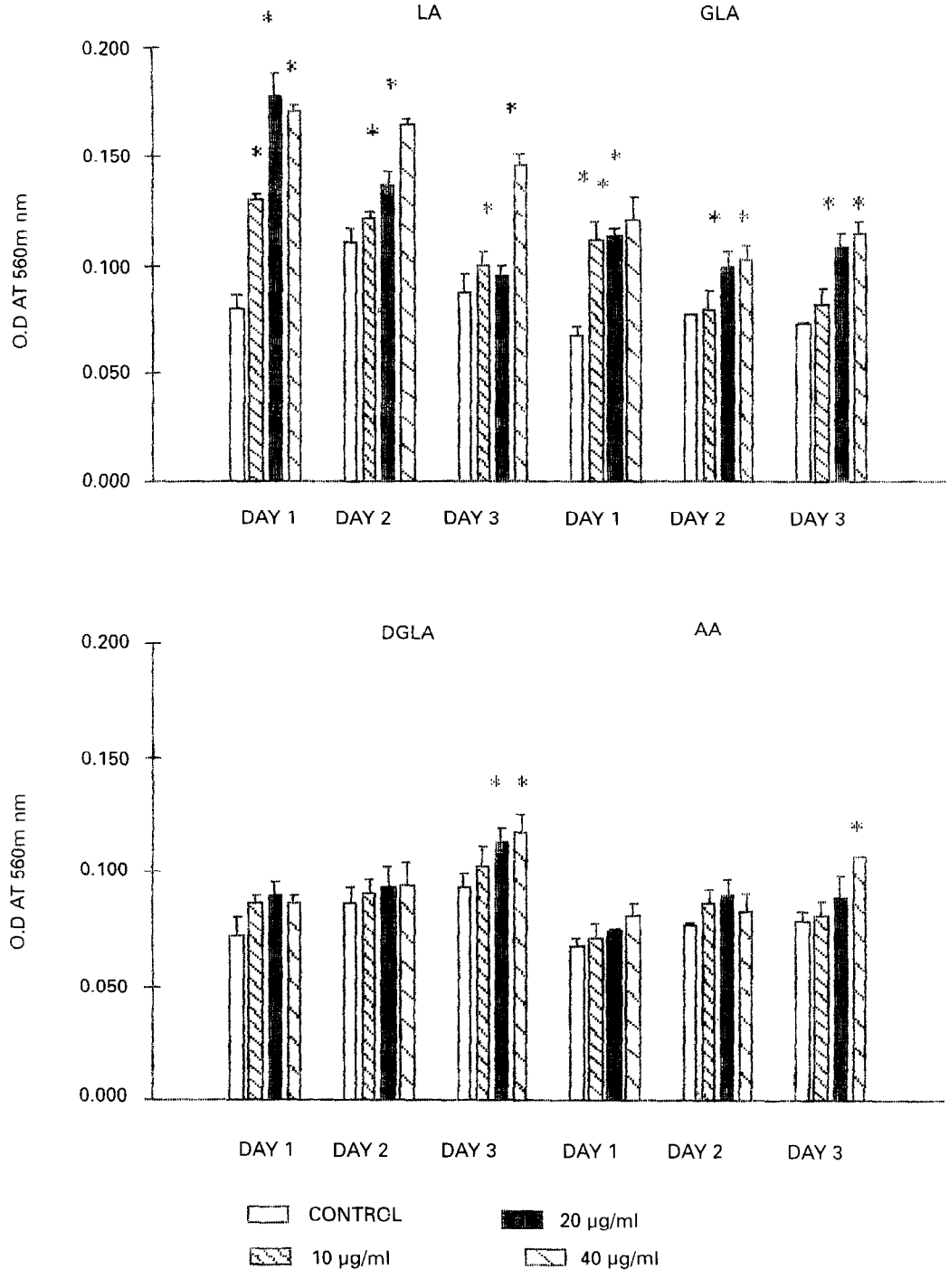

Fig. 3 Effect of fatty acids on superoxide generation. Superoxide anion was determined by Nitroblue Tetrazolium method. Briefly lymphocytes were incubated with 0.1% NBT dissolved in PBS, pH 7.4 for 1 h at 37°C. The reaction was terminated by adding 0.6 mL of glacial acetic acid into which the reduced NBT was extracted and was read at 560 nm. *P ≤ 0.05 compared to control.

acid and of their metabolites gamma-linolenic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, and of prostaglandins A and E on the proliferation of human osteogenic sarcoma cells in culture. Prostaglandins Leukot Med 1984; 15: 15–34.

). Jett M, Alving C R. Selective cytotoxicity of tumor cells induced by liposomes containing plant phosphotidylinositol. Biochem Biophys Res Commun 1983; 114: 863–871.

11. Seigel I, Lin T L, Yaghoubzadah E et al. Cytotoxic effects of free fatty acids on ascites tumor cells. J Natl Cancer Inst 1987; 78: 271–277.
12. Tolnai S, Morgan J F. Studies on the in vitro anti-tumor activity of fatty acids. V. Unsaturated fatty acids. Can J Biochem Physiol 1982; 40: 869–875.
13. Das U N, Padma M, Sangeetha Sagar P, Ramesh G, Koratkar R. Stimulation of free radical generation in human leukocytes by various stimulants including TNF is

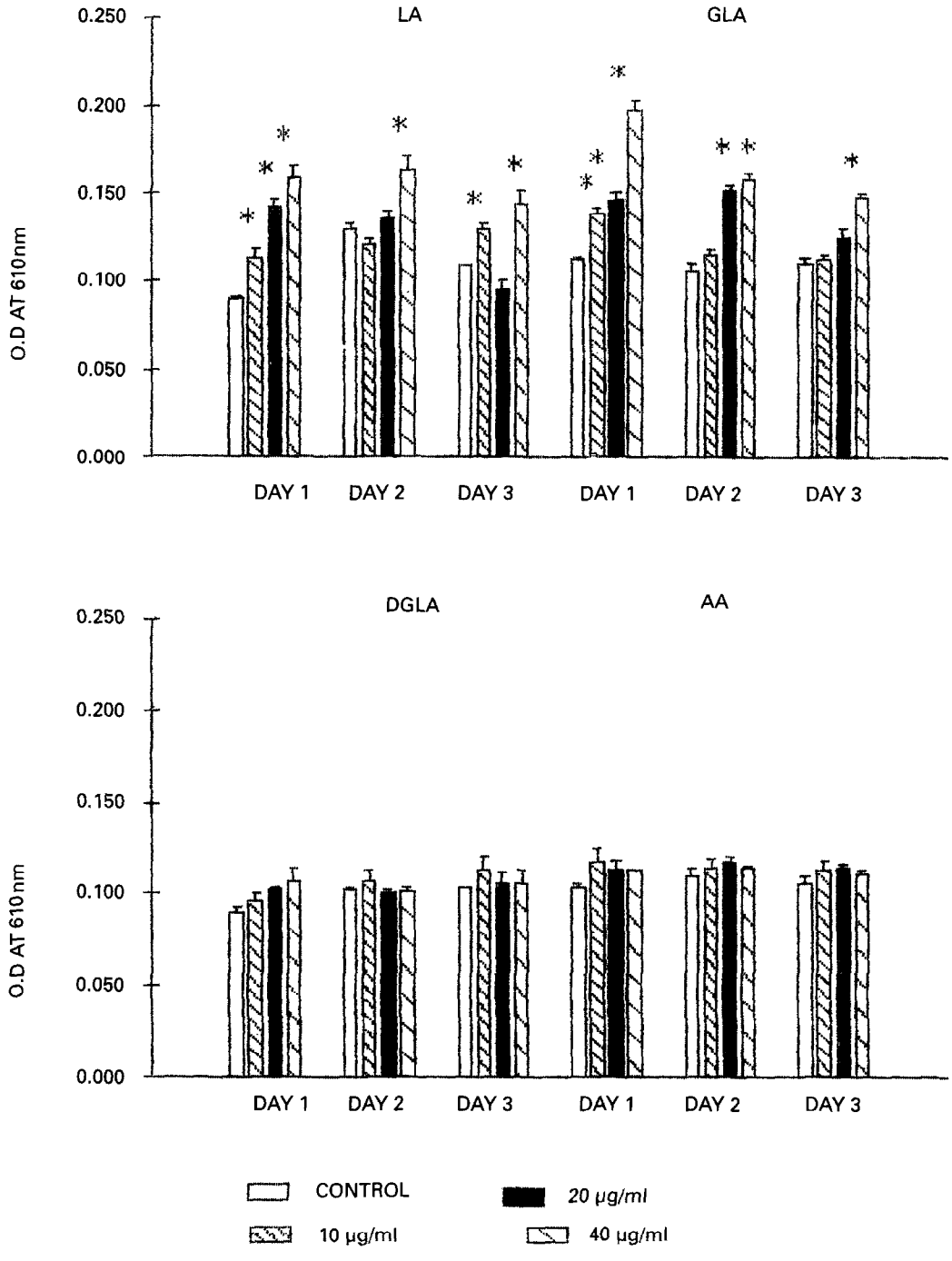

Fig. 4 Effect of fatty acids on H_2O_2 generation. The amount of hydrogen peroxide released is estimated by the horse radish peroxidase method. To the lymphocytes (1×10^6 cells/assay), 0.5 mL of Phenol Red solution (1%) was added and incubated at 37°C for 1 h. The reaction was terminated by adding catalase (150 U/assay) and absorbance was measured at 610 nm after making the assay mixture alkaline by adding 10 uL of 1 N NaOH. *$P \leq 0.05$ compared to control.

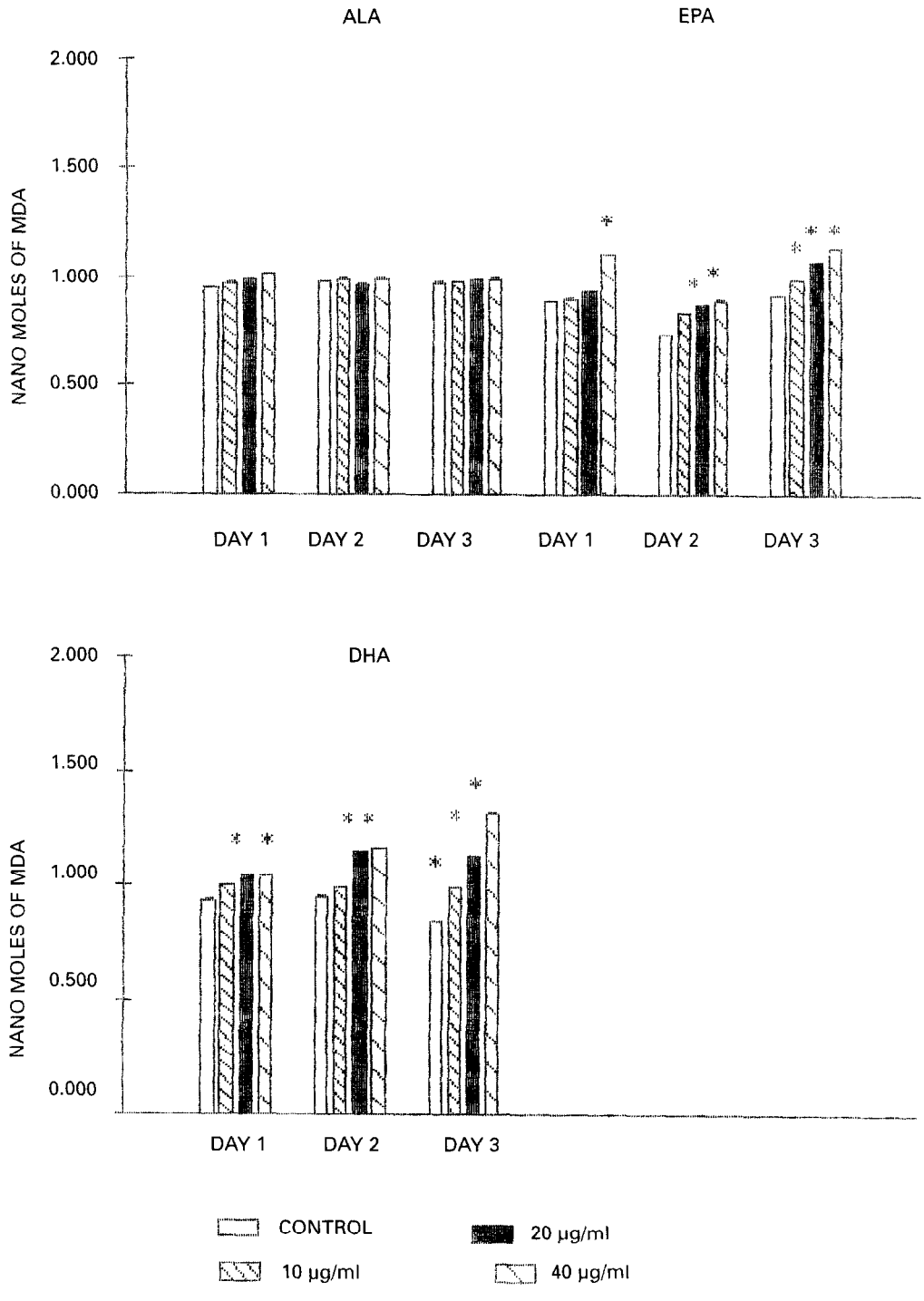

Fig. 5 Effect of fatty acids on lipid peroxidation. The total amount of lipid peroxidation products was estimated using the thiobarbituric acid (TBA) assay, which measures the malondialdehyde (MDA) reactive products. To 1 mL of trichloro acetic acid (TCA, 10%) treated cell supernatant 0.25 mL of TBA (0.33%) was added and boiled for 1 h at 95°C. After cooling the TBA reactive products were extracted in 1 mL of 1 N butanol and the intensity of the pink colour developed was read at 532 nm. *$P \leq 0.05$ compared to control.

a calmodulin dependent process. Biochem Biophys Res Commun 1990; 167: 1030–1036.
14. Das U N, Begin M E, Ells G, Horrobin D F. Polyunsaturated fatty acids augment free radical generation in tumor cells in vitro. Biochem Biophys Res Commun 1987; 145: 15–24.
15. Sangeeta P, Das U N, Koratkar R, Surya Prabha P. Increase in free radical generation and lipid peroxidation in patients with cancer following chemotherapy. Free Radical Biol Med 1990; 8: 15–20.
16. Santoli D, Zurier R B. Prostaglandin E precursor fatty acids inhibit human IL-2 production by a PGE-independent mechanism. J Immunol 1988 143: 1303–1309.