Abstract. There is now considerable evidence that polyunsaturated fatty acids (PUFA) are effective in vitro at limiting the growth of cancer cells while not affecting normal cells to the same extent. Twenty carbon PUFA, especially of the n3 series, have been shown to be particularly potent; while the eighteen carbon n6 fatty acid, linoleic acid has been implicated in growth stimulation of breast cancer. We report here on the comparative effects of a range of eighteen and twenty carbon fatty acids of varying degrees of unsaturation on normal and transformed fibroblasts in culture. All moieties of the n3 series showed high potency in limiting transformed cell growth, while cis and trans monounsaturates and pre-delta-6-desaturation n6 polyunsaturates induced a mixed response, even inducing comparative growth stimulation with some fatty acid concentrations.

Mammalian cells exhibit diverse abilities to desaturate dietary polyunsaturated fatty acids (PUFA) (1). The two major series of PUFAs (n6 and n3) are derived from plant-produced C18 precursors, the essential fatty acids (EFAs) linoleic acid (cis-C18:2n6, LA) and alpha-linolenic acid (cis-C18:3n3, ALA). The primary step in this process is the insertion of a further unsaturation between the n12 and n13 carbons, which reaction is catalysed by delta-6-desaturase. This is the first and rate-limiting step in the desaturase cascade which ultimately produces cis-C22:5n6 and cis-C22:6n3 (docosahexaenoic acid, DHA). Intermediates in the cascade include the metabolically important PUFA cis-C18:3n6 (gamma-linolenic acid, GLA), cis-C20:3n6 (dihomogamma-linolenic acid, DGLA), cis-C20:4n6 (arachidonic acid, AA) and cis-C20:5n3 (eicosapentaenoic acid, EPA). In the 1980's, evidence was published for impaired PUFA metabolism in transformed (cancer) cells, as well as some instances of fatty acids inhibiting cancer cell growth in culture (2, 3). During the subsequent two decades, a considerable body of literature has appeared describing the influence of PUFAs on cancer cells in vitro and in vivo, and there have been some attempts to utilise PUFAs, or their derivatives, in the treatment of certain cancers (4-8).

Even given the large body of evidence so far published on fatty acid involvement in cancer cell growth, there is still no definitive proof of how they induce their effects. Overall most PUFAs inhibit cancer cell growth, both in vivo and in vitro, but this pattern has not been seen in all cases. The observation of the stimulation of the growth of certain cancers, particularly breast and prostate in vivo, has complicated the picture somewhat (8, 9).

Most of the growth stimulatory effects have been shown with linoleic acid, both in vivo and in vitro, and this has led some to doubt whether PUFAs have real potential for use as effective anticancer agents (10, 11). In most instances, PUFAs are inhibitory of transformed cell growth, those of the n3 family of compounds appearing particularly potent, although they have the drawback of inhibiting normal cell division in some cases as well (12, 13). Interestingly, one of the most powerful growth inhibitors is the immediate delta-6-desaturase product from linoleic acid, gamma-linolenic acid, in which the only structural difference is the introduction of a third double bond between the n12 and n13 carbon atoms. Since all n3 PUFA already contain three methylene interrupted double bonds, and this is the difference between linoleic and gamma-linolenic acids, it may be this feature which confers their potency. In the past, attempts to elucidate a single, consistent mechanism were unconvincing, but recently the relationship of PUFAs with peroxisome proliferator activated receptors (PPARs) has shown promise of being a credible mechanism of action (14, 15).
Earlier studies in this laboratory have shown that monounsaturated fatty acids (MUFAs) and n6 PUFA pre-delta-6-desaturation, with either cis or trans double bond configurations, stimulated the growth of a mouse myeloma cell line in culture, but only within a limited range of concentrations (3). With all other concentrations the effects were inhibitory. In this study, the influence of a range of fatty acids of various degrees of unsaturation and of between eighteen and twenty-two carbons on the growth of normal human skin fibroblasts (HSF) and a fibroblast-derived, transformed cell line (3T6D) in culture was examined.

**Materials and Methods**

Two adherent fibroblast cell lines were used in this study: normal skin fibroblasts (HSF) and transformed fibroblasts (3T6D). Cells were seeded in 24-well tissue culture plates at 2 x 10⁴ cells per well, in 1 ml of medium comprising 90% DMEM and 10% fetal bovine serum. The cultures were incubated for 24 hours post-trypsinisation prior to dosing with fatty acids. Each of the fatty acids were individually dosed to cells at concentrations between 0 and 60 mg/l final culture volume, in increments of 10 mg/l, and using albumin as carrier (16).

The fatty acids used were cis-C18:1n9, trans-C18:1n9, cis-C18:2n6, trans-C18:2n6, cis-C18:3n3, cis-C18:3n6, cis-C20:2n6, cis-C20:3n3, cis-C20:3n6, cis-C20:4n6, cis-C20:5n3 and cis-C22:6n3.

The cultures were maintained for a further 48 hours after dosing at 37°C in an atmosphere of 5% CO₂:95% air. Subsequently, the cells were trypsinised and viability assessed using the Trypan Blue Exclusion method (17).

**Results**

The sample number (n) was 30 for each fatty acid with each concentration and with each cell line. Error bars have been omitted from both Figure 1 and Figure 2 for the sake of clarity. However, significant differences were shown (p<0.05) between HSF and 3T6D with trans-C18:1n9 (30, 40, 50 and 60 mg/l fatty acid dosed), trans-C18:2n6 (40, 50 and 60 mg/l), cis-C18:3n6 (50 and 60 mg/l), cis-C20:4n6 and cis-C20:5n3 (40, 50 and 60 mg/l).
Figure 1 shows the results of incubating the HSF cells with the fatty acids. All of the fatty acids, whether cis or trans, or MUFA or PUFA, had similar effects on the HSF cells. None of the fatty acids induced any growth stimulation with any concentration. Both cis-C20:3 and cis-C20:5 from the n3 series and cis-C20:4n6 caused very low levels of cell survival at high concentrations (50 and 60 mg/l).

Figure 2 shows the influence of the fatty acids on the 3T6D cells. cis-C20:2, cis-C20:3 and cis-C20:4 of the n6 series had only limited effects, with final percent viabilities for all three PUFAs above sixty percent. In comparison, cis-C18:3n6 induced the greatest degree of cell death, with only five percent surviving at the 60 mg/l dose. With cis-C18:1n9, cis-C18:2n6, trans-C18:2n6 and cis-C20:2n6, peaks of cell growth stimulation, compared to the underlying trend towards cell death, were observed. None of these fatty acids induced cell growth above the initial cell number, and the peaks of cell growth stimulation were all observed with a fatty acid concentration of 30 mg/l.

Discussion

The finding that the trans fatty acids (trans-C18:1n9, trans-C18:2n6) induced more cell death with the 3T6D cells is interesting, given the increased amounts of these fatty acids now seen in food products, especially margarines, and may indicate a role for these moieties in modulating the growth of transformed skin fibroblasts. In contrast, their cis equivalents (cis-C18:1n9, cis-C18:2n6) showed no overall difference in influence between the HSF and 3T6D cells, except for the peaks of cell growth; thus, olive oil may not be advantageous as far as skin fibroblasts are concerned. The dramatically negative effect of cis-C20:4n6 on the normal cells and almost negligible influence on the transformed cells could relate to the role of arachidonic acid as a 2-series eicosanoid precursor, or may reflect an impact of the fatty acid itself directly. In either case, it does show a need to moderate the intake of this PUFA, particularly common in red meat, when transformation of skin cells is a concern. Similarly, but not to the same degree, cis-C20:5n3, common in fish, also had a greater negative influence on the HSF than the 3T6D cells. The PUFA moiety with the least negative influence on the HSF cell line, and greatest on the 3T6D cells, was cis-C18:3n6, gamma-linolenic acid, yet its immediate precursor, cis-C18:2n6, and immediate product, cis-C20:3n6, were considerably less effective or selective. This indicates a susceptibility of transformed fibroblasts to a trienoic fatty acid, but of a specific carbon chain length only, and a comparative absence of that susceptibility in normal skin fibroblasts. If that susceptibility were linked to the eicosanoid derivatives of PUFA, then one would expect the cell killing capability to increase when the eicosanoid precursor PUFA were dosed, but this was not the case.

Recently, there have been some indications that conjugated fatty acids, especially conjugated linoleic acid (CLA), may be effective limiters of cancer cell growth (18). CLA has several forms, all of which have had one or both of the cis unsaturations converted to the trans configuration. Thus, it is possible that the trans-C18:1n9 and trans-C18:2n6 in this study may work via a similar mechanism. Unfortunately, we were not able to obtain CLA to allow us to assess its impact on our system.

At present, we do not understand the mechanisms underlying the peaks of cell growth limitation reversal seen with cis and trans-C18:1n9, cis and trans-C18:2n6 and cis-C20:2n6, but measurement of peroxidation of the fatty acids by assay of the MDA-equivalents indicated no comparable increase or decrease in these products coincident with the peaks (data not shown), and thus this is not considered a likely explanation (16).

All the moieties showing the capability to induce these peaks were pre-delta-6-desaturation, and with a maximum of two unsaturations, which may be significant in understanding the mechanisms operating within skin fibroblasts, especially when they become transformed.

However, circulating fatty acids are never found in isolation since lipids contain a variety of fatty acids with various degrees of unsaturation within individual lipid molecules. This means that cells in vivo are unlikely to be exposed to high concentrations of any specific fatty acid, so one must be cautious in extrapolating the results from individual fatty acids.

References


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