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Evaluation of the Effect of Fish Oil on Cell Kinetics: Implications for Clinical Immunosuppression

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FISH OIL is known to exert salutary effects on cell proliferation in several tissues, and inhibitory effects against inflammation and the response to injury. We evaluated the effect of fish oil on cell cycle kinetics in cultured human Hs68 fibroblasts, for defining its potential use as an immunosuppressive agent.

METHODS

Cell Culture and Fatty Acid Treatment

Exponentially growing fibroblasts were cultured for 5 days in regular media (CNT) and in media containing 50 μg/mL fish oil (FO) or corn oil (CO) in the presence and absence of the cyclooxygenase inhibitor indomethacin (IND). For treatment with extrinsic fat, cells were plated in growth medium for 24 hours, after which the medium was replaced with fresh oil-enriched medium at a final oil concentration of 50 μg/mL. The medium was changed daily to decrease the reaction of fatty acid oxidation on cells. After 5 days in culture, cells were harvested by trypsinization and reseeded into 100-mm tissue culture dishes.

Bromodeoxyuridine (BrdUrd) Pulse Labeling and Staining Procedure for Cell Cycle Kinetics

Following 5-day oil treatment, cells were reseeded to reestablish exponential growth characteristics. The cells were then fixed and stained for bivariate BrdUrd/DNA flow-cytometric analysis as previously described.[1]

Flow Cytometry

The cells were analyzed with FASCAN (Becton-Dickinson, Mountain View, Calif), with excitation light intensity at 488 nm and 15 mW.

Kinetic Analysis

Cell cycle kinetic parameters were determined by use of BrdUrd pulse labeling and bivariate BrdUrd/DNA analysis by flow cytometry. Bivariate BrdUrd/DNA histograms allow identification of G1/G0 and G2/M cells according to DNA content.

Labeled S-phase cells were identified by their BrdUrd uptake and separated into undivided and divided subgroups These fractions were used to calculate potential doubling time and cell cycle kinetics, as previously described.[2]

RESULTS

The results of cell cycle kinetics are shown in Table 1.

The data suggest an effect of fish oil on the S phase of cell division. After 5 days of treatment, S-phase distribution was significantly increased as a result of lengthening of the S phase for FO fibroblasts compared with CO and CNT. This effect appears to be independent of prostaglandin metabolism as noted by the response to IND treatment.

DISCUSSION

Fish oil acts on the S phase of cell division. This effect is unique because major immunosuppressive drugs, like cyclosporine and tacrolimus, act on G0, early in the cell cycle. Fish oil could have important synergistic properties with other immunosuppressive drugs, especially when used in combination with other anticytokine immunosuppressive agents. Furthermore, the nonnephrotoxic property of fish oil makes it quite attractive for use as an adjuvant immunosuppressive agent with calcineurin inhibitors, which are

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Table 1. Effects of Fish Oil and Corn Oil on Cell Cycle Kinetics of Exponentially Growing Fibroblasts

Treatment	% G1/G0	% S	% G2/M	Labeling Index	T_S (Hours)
CNT	68 (1)	25 (1)	7 (0)	0.19 (0.00)	6.6 (0.2)
CO	68 (1)	24 (0)	7 (0)	0.19 (0.00)	6.6 (0.3)
FO	58 (2)*	33 (1)*	9 (0)	0.26 (0.00)*†	9.1 (0.9)§
FO + IND	61 (2)*	31 (0)*	9 (1)	0.22 (0.00)	8.0 (0.4)
CO + IND	69 (0)	21 (0)	9 (0)	0.16 (0.00)‡	8.3 (0.2)

Data expressed as means (SEM) (n = 8). T_S, S-phase duration (hours).
*$P < .0001$ vs CO, CNT, and CO+IND; †$P < .01$ vs FO + IND; ‡$P < .005$ vs CO and CNT; §$P < .004$ vs CO and CNT.

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known to be associated with significant nephrotoxicity. The unique effect of fish oil in increasing the S-phase duration of dividing fibroblasts suggests a potential role for preventing chronic rejection, which deserves further investigation.

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