in vivo *21*: 333-338 (2007)

The Influence of Shark Liver Oils on Normal and Transformed Mammalian Cells in Culture

B.C. DAVIDSON[1], D. ROTTANBURG[1], W. PRINZ[1] and G. CLIFF[2]

[1]*School of Physiology, University of the Witwatersrand Medical School, Johannesburg;*
[2]*Gauteng and Natal Sharks Board, Umhlanga Rocks, KwaZulu-Natal, South Africa*

Abstract. *n3 polyunsaturated fatty acids have been reported to have anti-carcinogenic effects on mammalian carcinomas. n3 fatty acids occur in high concentrations in marine oils, especially shark liver oils. Several reports have indicated an extremely low incidence of cancer in sharks, although other reports indicate carcinogenesis in some shark species. It has been hypothesised that n3 fatty acids and other components of shark liver oil may exert anti-carcinogenic effects. The aim of this study was to assess whether shark liver oil, from four Indian Ocean shark species, exerted anti-proliferative effects on transformed and normal mammalian cells in culture, and to assess whether the ratio of n3 to n6 polyunsaturates influenced the results. Neither the shark liver oils themselves, nor the ratio of n3 to n6, showed any consistently significant effects with either transformed or normal cells.*

For several years polyunsaturated fatty acids, long chain fatty acids with two or more double bonds and the first double bond in either the n3 (omega-3) or n6 (omega-6) position, have been suggested as anti-carcinogenic agents (1-8). They not only form important components of cell membrane phosphoglycerides (9), but are reported to have a number of health benefits in humans (10). Anti-inflammatory, anti-proliferative and anti-carcinogenic effects have been demonstrated with n3 fatty acids and their derivatives (11). Both *in vivo* and *in vitro* studies have shown that n3 fatty acid supplementation inhibits the promotion and progression stages of carcinogenesis (12), increases the efficacy of various anticancer drugs, chemotherapy and radiation, as well as aiding in the correction of cachexia (13). In contrast, n6 fatty acids and their derivatives have both anti- and pro-inflammatory effects, and stimulate cell proliferation of some cell types, as well as increasing the incidence of certain tumours, particularly mammary (11). As a result of the differing effects of n3 and n6 fatty acids on cell growth, the n3:n6 ratio is considered important for normal metabolic functioning as well as in carcinogenesis (10), and modulation of the n3:n6 ratio by increasing the amount of n3 fatty acids has been reported to have a beneficial role in anti-carcinogenesis of breast cancer (14). Most studies indicate that a negative correlation exists between the n3:n6 ratio and cancer mortality (15), thus suggesting that the higher the n3:n6 ratio, the greater the anti-proliferative and anti-carcinogenic effect.

Neither n3 nor n6 fatty acids can be synthesised by mammals and thus must be obtained from the diet. The greatest concentration of n3 fatty acids found naturally occurs in marine oils, such as shark liver oils (13). Shark liver oil administered to humans has been shown to elevate white blood cell counts thereby strengthening the immune system (16), to reduce the side effects of radiation (10, 17), as well as inhibiting tumour angiogenesis (18).

Most shark liver oils examined to date contain a range of lipid classes, including alkylglycerols, triacylglycerols, squalene and fatty acids (mostly n3 polyunsaturated fatty acids, but also saturated, monounsaturated and n6 polyunsaturated fatty acids) (16). However, the composition of shark liver oils differ between shark species, and has been shown to be dependant on shark size, sex, diet, growth rate, swimming depth and reproductive status, as well as the season (17, 19). Variation in lipid composition may also be related to the rate limits of the biosynthesis of shark liver oil components (17).

Initially, several reports suggested that sharks did not develop cancer (20, 21). However, others found malignant and benign tumours in 42 sharks distributed across 21 species, although the number of individual sharks examined, and, thus, the absolute incidence, was not reported (22). Failure to induce tumours in shark tissues *in vitro* has been taken as evidence of the resistance of sharks to carcinogenesis (20, 21). In addition, some authors

Correspondence to: Prof Bruce Davidson, School of Physiology, University of the Witwatersrand Medical School, Johannesburg, 2193, South Africa. Tel: +27 11 717 2464, Fax: +27 11 643 2765, e-mail: davidsonbc@physiology.wits.ac.za

Key Words: Shark liver oil, fatty acids, cancer cells.

suggest that the high levels of polyunsaturates, especially n3 polyunsaturates, in shark liver oils may be of benefit in retarding the growth of cancers, not only in sharks themselves, but also in mammals (18). The aim of this study was to assess whether shark liver oils from different Indian Ocean shark species exert anti-proliferative effects on both transformed and normal mammalian cells *in vitro*, across a range of oil concentrations reflecting fatty acid concentrations that occur in mammalian circulation under normal physiological conditions.

Materials and Methods

The cancer cell lines used in the study were Caco2 (human colon adenocarcinoma) and 653 (P3X63.Ag8.653 mouse myeloma). Normal cell lines used in the study were 3T3 (immortalized mouse fibroblasts), and MRC5 (human lung embryonic cells). The Caco2, 3T3 and MRC5 cell lines were obtained from Highveld Biologicals (Pty) Ltd., South Africa. The 653 cell line was obtained from Dr. W. Prinz of the University of the Witwatersrand, Johannesburg, South Africa. The culture media used were modified Eagle's medium (MEM) for the Caco2 cell line, Rosewell Park Memorial Institute medium (RPMI) for the 653 cell line, Dulbecco's modified Eagle's medium (DMEM) for the 3T3 cell line and Leiberwitz medium for the MRC5 cell line. Each of the media contained 10% foetal calf serum and 2% Penstrep (an antibiotic mixture containing both penicillin and streptomycin). The foetal calf serum, media and Penstrep were all obtained from Highveld Biologicals (Pty) Ltd., South Africa.

The shark liver samples were obtained from the Natal Sharks Board, KwaZulu Natal, South Africa. Each shark caught was identified, sexed, measured and recorded. Live sharks were tagged and released, whereas dead sharks were weighed and dissected. Samples of liver tissue were removed and frozen at –20˚C prior to oil extraction at the University of the Witwatersrand. The shark liver oil was extracted and purified according to the procedure of Bligh and Dyer (1959) and stored at –20˚C. Prior to dosing of the cells, any contaminants were removed by microfiltration. Since the fatty acid profiles of sharks differ, shark liver oils from four different species of shark were used, namely: dusky (*Carcharhinus obscurus*), spinner (*Carcharhinus brevipinna*), spotted raggedtooth (*Carcharias taurus*) and great white (*Carcharodon carcharias*). The shark liver oils were solublised with bovine serum albumin (BSA) - fraction V. The albumin itself had no effect on cell viability (data not shown). The shark liver oil/BSA solutions were prepared to give final concentrations in the culture plate well of 0, 40, 80, 120, 160 and 200 mg oil/l culture medium.

Each cell line was grown in 75 cm^2 or 150 cm^2 flasks in the appropriate culture medium and were incubated at 37˚C. Cells were removed from the flasks using brief trypsinisation where necessary. Cells in medium were then seeded into 24 well tissue culture plates at $2x10^5$ cells per well in 1 ml of medium, and left for 24 hours to recover prior to oil dosing. One hundred μl of the shark liver oils were then individually dosed to each well over the concentration range listed above. A sample size of six was obtained for each cell line per shark where possible, however slow MRC5 cell growth meant insufficient numbers were obtained for a sample size of six, and a sample size of four was used. Also, insufficient numbers of the 3T3 cell line could be grown during the time window available for this study, and only the ragged tooth and spinner shark liver oils were used. After dosing culture plates were incubated for 48 hours, which has previously been shown to be optimal (1, 25). Cell viability was assessed by the Trypan Blue Exclusion method (26).

Repeated measures analysis of variance (ANOVA) with Dunnett's *post hoc* test was used to compare the various concentrations of shark liver oils with each of the cell lines. In all cases significance was assumed when $p<0.05$.

Table I. *The total lipid and fatty acid composition of the shark liver oils.*

Fatty acid	Dusky	Great White	Raggedtooth	Spinner
12:0	-	0.03	0.05	0.04
14:0	9.35	4.86	6.69	5.13
16:0	13.05	15.61	16.45	16.70
18:0	3.04	4.12	3.36	9.32
TSFA	25.44	24.62	26.55	31.19
14:1n9	-	1.24	0.68	1.08
16:1n9	20.43	15.63	13.28	7.41
18:1n9	24.21	33.49	21.44	15.95
20:1n9		0.84	2.09	-
TMUFA	44.64	50.36	37.49	24.44
16:2n6	-	1.86	0.97	0.53
18:2n6	-	-	-	1.21
20:3n6	-	-	0.03	0.85
20:4n6	1.17	1.29	3.20	5.59
22:4n6	-	0.41	0.58	1.28
22:5n6	0.12	0.33	0.86	1.88
Tn6PUFA	1.29	2.03	4.67	11.34
18:3n3	3.87	0.96	1.47	1.79
18:4n3	-	-	-	0.63
20:3n3	-	-	-	0.18
20:5n3	13.68	4.44	9.92	8.15
22:5n3	-	2.07	15.57	4.21
22:6n3	10.65	12.30	2.37	14.80
Tn3PUFA	28.20	19.77	29.33	29.76
TPUFA	29.49	21.80	34.00	41.10
n3:n6	21.86	9.74	6.28	2.62

TSFA = total saturated fatty acids; TMUFA = total monounsaturated fatty acids; Tn6PUFA = total n6 polyunsaturated fatty acids; Tn3PUFA = total n3 polyunsaturated fatty acids; TPUFA = total polyunsaturated fatty acids; n3:n6 = ratio of n3 to n6 polyunsaturated fatty acids.

Results

The fatty acid composition of the dusky, great white, raggedtooth and spinner liver oils are shown in Table I.

Figure 1 shows the impact of dusky liver oil on the cell types used in this study. The growth of Caco2 cells showed an overall trend to growth increase, but the scatter was large, and the differences were not significant at $p<0.05$. Similarly there were no significant differences with MRC5 cells, and only the 200 mg/l concentration of dusky liver oil induced a slight, but significant ($p<0.05$), increase in

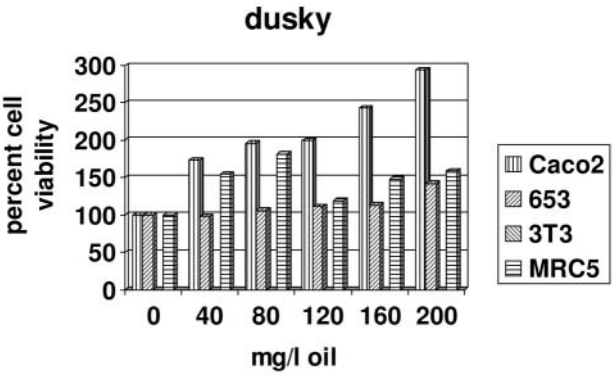

Figure 1.

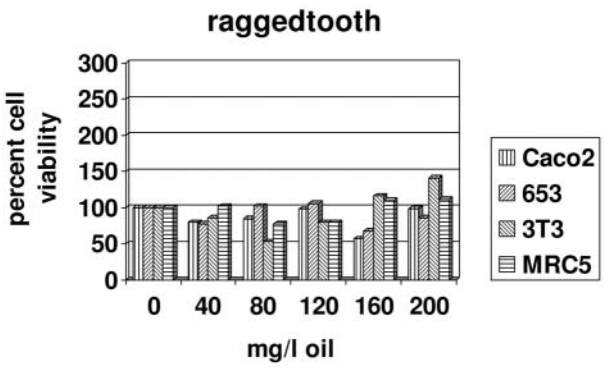

Figure 3.

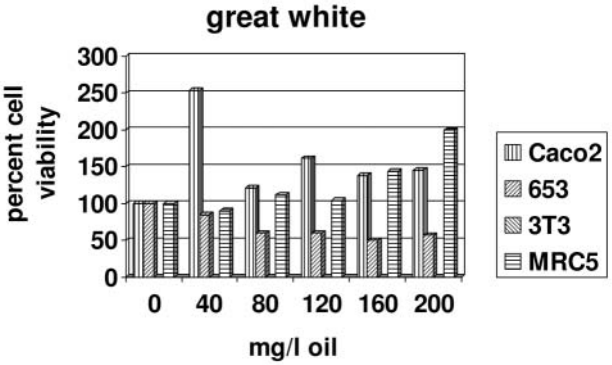

Figure 2.

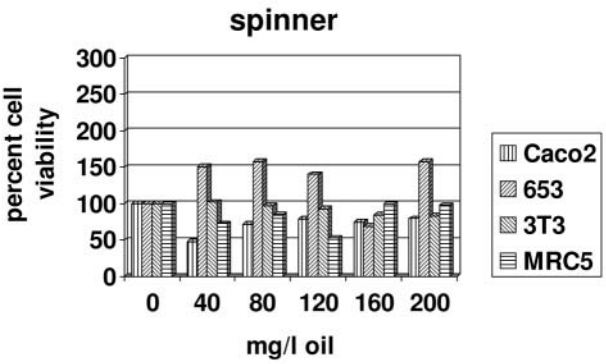

Figure 4.

Figures 1-4 show the impact of the four shark liver oils on the four cell types used in this study. Results are expressed percent cell viability versus shark liver oil concentration in the culture medium. Error bars have been omitted for the sake of clarity. Significant differences are indicated in the text.

653 cell growth. 3T3 cells were not grown with this shark liver oil.

Figure 2 shows the impact of great white liver oil on the cell types used in this study. There were no significant differences in cell growth with any of the shark oil concentrations or any of the cell types used. 3T3 cells were not grown with this shark liver oil. There was a trend towards cell growth increase with Caco2 cells, especially at 40 mg/l, and for MRC5 cells with higher concentrations of oil, but not significant. The 653 cells showed a trend towards growth inhibition, but again this was not significant.

Figure 3 shows the impact of raggedtooth liver oil on the cell types used in this study. There were no significant differences in cell growth with any of the shark oil concentrations or any of the cell types used. 3T3cells showed growth inhibition with lower concentrations of this oil, which trend reversed with higher concentrations. 160 mg/l caused a decrease in cell growth of Caco2 cells, but again not significant.

Figure 4 shows the impact of spinner liver oil on the cell types used in this study. There was a trend towards growth inhibition with low concentrations with CacO2 cells. The converse was true for 653 cells, and with oil concentrations of 40 mg/l, 80 mg/l and 200 mg/l this growth stimulation was significant ($p<0.05$).

Discussion

The objective of the study was to determine whether shark liver oils exerted anti-proliferative effects on cancer *versus* normal cells in culture. The results of the study were inconsistent: all of the normal cell lines and most of the cancer cell lines, showed no statistically significant effects on cell growth in response to the various shark liver oils. However, some of the cancer cell lines did exhibit either stimulation or inhibition of cell growth with some oil concentrations. But there was no overall significant trend in the influence of the various shark liver oils on the cancer and normal cell lines.

The dusky shark liver oil stimulated 653 cell growth (Figure 1), but had no significant effect on any of the other cell lines used in the study. The n3:n6 ratio of the dusky shark liver oil and free fatty acid mixture was relatively high (Table I), therefore it was expected for the oil to exert inhibitory effects on cell growth, however this was not so.

The great white shark liver oil induced the greatest inhibitory effect at all concentrations on the 653 cell line but had no significant effect on the other cell lines used (Figure 2). The great white shark liver oil n3:n6 ratio was only moderate, suggesting it should have exerted the minimal effects, but the converse was the case.

The raggedtooth shark liver oil had no significant effect on any of the cell lines (Figure 3). Because of its intermediate n3:n6 ratio, the raggedtooth shark liver oil (Table I) was expected to have only limited effects on cell growth, and this was indeed the case.

The spinner shark liver oil exerted the greatest stimulatory effect on the growth of the 653 cell line (Figure 4), but had no significant effect on any of the other cell lines tested. As previously stated, other studies have shown that the amount of n3 and n6 fatty acids may have an influence on carcinogenesis (10). As a result of the relatively low n3:n6 ratio (Table I) of spinner shark liver oil, one would have expected the oil to have the least anti-proliferative effects, and possibly proliferative effects, on growth of the different cell lines, but with the one exception noted above this was not the case.

One possible explanation for the lack of impact of the oils was the presence of other components of shark liver oil that could have influenced cell proliferation, but given the highly complex nature of a natural oil, this could not be assessed.

The literature shows a high n3:n6 ratio to be associated with tumour suppression (14), but overall our results do not confirm this, and suggest that a high n3:n6 alone is not sufficient to predict the efficacy of a shark liver oil in suppressing tumour development. These findings also concur with a study which showed that increasing the n3:n6 ratio from 0.01 to 7.80, resulted in tumour development rather than tumour suppression in some instances (12).

Extensive literature reports on the beneficial effects of alkylglycerols, a component of shark liver oils found in species from colder waters (27). However, the shark liver oils used in this study were from Indian Ocean shark species, and exhibited only trace amounts of alkylglycerols. This marked difference in composition may underly the apparently contradictory results obtained, and motivate for caution in extrapolation of data from different marine environments, even if from the same species.

Acknowledgements

The authors would like to thank the National Research Foundation of South Africa and the University of the Witwatersrand, Johannesburg, South Africa for financial support of this project.

References

1 Giangregorio A: The influence of fatty acids *in vitro* on mammalian cells from species differing in their fatty acyl desaturase capabilities. Ph.D. Thesis, University of the Witwatersrand, Johannesburg, South Africa, 1992.

2 Cantrill RC, Davidson BC, Katzeff I and Booyens J: The effects of essential fatty acid supplementation on the fatty acid composition of cancer cells in culture. Prog Lipid Res *25*: 547-550, 1986.

3 Girao LA, Ruck AC, Cantrill RC and Davidson BC: The effect of C18 fatty acids on cancer cells in culture. Anticancer Res *6*: 241-244, 1986.

4 Davidson BC, Girao LAF, Giangregorio A and Murphy J: Polyunsaturated fatty acids modulate fibroblast growth in culture. Anticancer Res *11*: 267-272, 1991.

5 Davidson BC, Giangregorio A and Girao LAF: The influence of C18 fatty acids on the growth of fibroblasts of different degrees of transformation in culture. Anticancer Res *13*: 795-800, 1993.

6 Davidson BC, Giangregorio A and Girao LAF: The influence of fatty acids on normal and transformed human liver cells in culture. Anticancer Res *18*: 3533-3538, 1998.

7 van der Merwe CF, Booyens J and Katzeff IE: Oral gamma-linolenic acid in 21 patients with untreatable malignancy – an ongoing pilot open clinical trial. Br J Clin Pract *41*: 907-915, 1987.

8 Davidson BC, Girao LAF and Giangregorio A: Differential influence of pre- and post-delta-6-desaturation n6 polyunsaturated fatty acids on fibroblasts in culture, compared to n9 monounsaturated and n3 polyunsaturated fatty acids. In Vivo *19*: 221-224, 2005.

9 Heidmann-Soccol MC and Oetterer M: Seafood as a functional food. Braz Arch Biol *7-46*: 443-454, 2003.

10 Jayasinghe C, Gotoh N, Tokairin S, Ehara H and Wada S: Inter species changes of lipid compositions in liver of shallow-water sharks from the Indian Ocean. Fisheries Science *69*: 644-653, 2003.

11 Larsson SC, Kumlin M, Ingelman-Sundberg M and Wolk A: Dietary long chain n3 fatty acids for the prevention of cancer: a review of potential mechanisms. Am J Clin Nutr *79*: 935-945, 2004.

12 Sasaki T, Kobayashi Y, Shimizu J, Wada M, In'nami S, Kanke Y and Takita T: Effects of dietary n3-to-n6 polyunsaturated fatty acid ratio on mammary carcinogenesis in rats. Cancer *30*: 137-143, 1998.

13 Hardman WE: (n3) Fatty acids and cancer therapy. J Nutr *134*: 3427S-3430S, 2004.

14 Man-Fan Wan J, Kanders BS, Kowalchuk M, Knapp H, Szeluga DJ, Bagley J and Blackburn GL: N3 fatty acids and cancer metastasis in humans. World Rev Nutr Diet *66*: 477-487, 1991.

15 Galli C and Butrum R: Dietary n3 fatty acids and cancer: an overview. World Rev Nutr Diet *66*: 446-461, 1991.

16 Solomon N, Passwater R and Joelsson I: Shark liver oil and cancer. *In*: Shark Liver Oil-natures Amazing Healer. New York, Kensington publishing company, pp. 49-54, 1997.

17 Wetherbee BM and Nichols PD: Lipid composition of the liver oil of deep-sea sharks from the Chatham Rise, New Zealand. Comp Biol Physiol B *125*: 511-521, 2000.

18 Skopinska-Rosewska E, Krotkiewski IM, Sommer E, Rogala E, Filewska M and Bialas-Chromeic B: Inhibitory effect of shark liver oil on cutaneous angiogenesis induced in Balb/c mice by syngeneic sarcoma L-1, human urinary bladder and human kidney tumour cells. Oncol Rep *6*: 1341-1344, 1999.
19 Navarro-Garcia G, Pacheco-Aguilar R, Vellejo-Cordova B, Ramirez-Suarez JC and Bolanos A: Lipid composition of the liver oil of shark species from the Caribbean and Gulf of California waters. J Food Compos Anal *13*: 791-798, 2000.
20 Lane IW: Sharks don't get cancer. Avery Publishing Group, New York, pp. 1-100, 1992.
21 Lane IW: Sharks still don't get cancer. Avery Publishing Group, New York, pp. 1-43, 1996.
22 Ostrander GK, Cheng KC, Wolf JC and Wolfe MJ: Shark cartilage, cancer and the growing threat of pseudoscience. Cancer Res *64*: 8485-8491, 2004.
23 Bligh EG and Dyer WJ: A rapid method of total lipid extraction and purification. Can J Biochem Physiol *37*: 911-917, 1959.
24 Kay MMB and Makinodan T: CRC Handbook of Immunology in Aging. CRC Press, Boca Raton, USA, pp. 30-40, 1981.
25 Girao LAF: To determine the *in vitro* effects of C18 fatty acids on normal, malignant and benign cell lines. Ph.D. Thesis, University of the Witwatersrand, Johannesburg, South Africa, 1988.
26 Paul J: Cell viability by Trypan Blue Exclusion. *In*: Cell and Tissue Culture. Churchill Livingstone Press, London, pp. 367-368, 1975.
27 Pugliese PT, Jordan K, Cederberg H and Brohult J: Some biological actions of alkylglycerols from shark liver oil. J Altern Complement Med *4*: 87-99, 1998.

Received September 5, 2006
Accepted September 14, 2006