THE STRUCTURE AND FUNCTION OF OMEGA-3 POLYUNSATURATED FATTY ACIDS IN MODEL AND CELLULAR MEMBRANES

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SUMMARY

Dissertation: The Structure and Function of Omega-3 Polyunsaturated Fatty Acids in Model and Cellular Membranes.

Epidemiology studies conducted in the early 1970s indicated that the ingestion of fish and fish oil may reduce the incidence of certain human health afflictions, including atherosclerosis and cancer. Later it was found that the molecule most responsible in alleviating these afflictions was a polyunsaturated fatty acid known as docosahexaenoic acid (DHA). DHA is a member of a class of polyunsaturated fatty acids known as omega-3s. This molecule is the most unsaturated fatty acid found in nature, and represents the extreme for monitoring the effects of polyunsaturation on membrane bilayer properties. The fact that DHA is the most unsaturated fatty acid, that each unsaturation requires energy to produce, and that nature will only produce molecules that have a specific function, led us to question the role of this molecule in the membrane bilayer. Specifically, why would nature produce a molecule, like DHA, if other less unsaturated fatty acids could provide the same function? The research presented here attempts to answer this question by monitoring the effects of DHA and other unsaturated fatty acids in model membranes. The results will then be compared to the effects of this molecule in processes thought to be important in: atherosclerosis, cancer and aging.

Model membranes composed of DHA added as fatty acid to a saturated membrane or esterfied to the sn-2 position of a phosphatidycholine were made and compared with other less unsaturated

fatty acids for the following properties: membrane fluidity, fusion, permeability and interaction with cholesterol. Model membrane fluidity was monitored by fluorescence polarization of a series of anthroyloxy stearic acid probes attached at the 2 (2AS), 6 (6AS), 9 (9AS) or 12 (12AS) positions on the stearic acid molecule. The results from these experiments indicate that while DHA and other unsaturated fatty acids increase membrane fluidity relative to totally saturated membranes, there was no detectable difference between membranes that contained DHA and less unsaturated bilayers. Further proof of this observation was provided by examining the polarization of 1,6-diphenyl-1,3,5-hexatriene probe (DPH), which yielded the same results as the AS probes.

Because DHA must have some function in the membrane we next examined the effects of this molecule on two other membrane properties:` membrane fusion and permeability. Resonance energy transfer data collected for vesicles composed of 80 mol% DMPC 20 mol% 22:6 or 18:0,22:6 PC small unilamellar vesicles (SUV) indicate that the presence of DHA perturbed bilayer structure leading to increased fusion relative to oleic, linoleic, α-linolenic, and γ-linolenic acids Similar results were obtained by an aqueous compartment mixing assay. The effects of DHA and other unsaturated fatty acids on model membrane permeability were monitored by movement of non-charged (erythritol permeability) and charged (carboxyfluorescein permeability) species. These results demonstrate that DHA enhanced bilayer permeability relative to less unsaturated fatty acids.

While DHA was clearly affecting fusion and permeability of model membranes composed of saturated PCs containing PUFAs or mixed chain PCs, we wanted to better understand the interaction of this molecule with

other biologically relevant lipids. Cholesterol, an important sterol that helps maintain a variety of membrane properties was chosen to better understand the interaction of this sterol with DHA and other PUFAs. Monolayers composed of a 1:1 mixture of cholesterol and mixed chain PCs containing in the sn-1 position stearic acid and in the sn-2 position either oleic, α-linolenic, γ-linolenic, or docosahexaenoic acid were made and tested for the effects of cholesterol on condensation (alterations in total area occupied by the sum of the lipids) of these two component monolayers. The results demonstrate that mixed chain PCs containing oleic or α-linolenic acid were condensable while those that contained γ-linolenic or DHA were not, indicating a preference for cholesterol location in the membrane based on acyl chain structure.

Next, the effects of DHA on rabbit HDL order and molecular dynamics was monitored by supplementing into the diet of rabbits menhaden oil (MO fed group, rich in omega-3s) or hydrogenated cottonseed oil (HCTO fed group, rich in saturated fat). Rabbit HDL was isolated and purified by differential flotation ultracentrifugation and then subjected to ESR of either 5- or 16- doxyl stearic acids to monitor outer monolayer order. The results indicated that neither the 5- or 16-doxyl probes could detect a difference between HDL from MO or HCTO fed rabbits. Inner core order was likewise monitored by ESR of cholesteryl 12-doxyl stearic acid and order parameters derived from fluorescence anisotropy of DPH. In contrast to the results obtained with the outer monolayer probes, inner core order was substantially different for the two experimental groups. The MO fed group had decreased HDL inner core order as detected by the ESR and fluorescent probes compared to the HCTO fed rabbits. These results indicate that the presence of DHA

decreases inner core order which may lead to increased cholesterol exchange and could decrease the likelihood of atherosclerosis.

The presence of DHA in the model membrane systems altered the permeability and condensation properties of the bilayer, which suggests that DHA could affect growing tumor cells by disrupting membrane structure. T27A, a non-B, non-T leukemia was used as a model system to examine the effects of DHA on tumor cell membrane fluidity, permeability and immunological domains. Tumor cell membrane fluidity was monitored by fluorescence polarization of the anthroyloxy stearic acid probes (2AS, 6AS, 9AS and 12AS). T27A plasma membranes altered by dietary exposure to menhaden oil (MO) or hydrogenated coconut oil (HCO) in BALB/c mice were isolated by centrifugation and labeled with the AS probes. Plasma membrane fluidity was not altered by the presence of DHA when compared to the HCO fed group. T27A membrane permeability was monitored by erythritol swelling and ^{51}Cr release. T27A cells were fused with either 18:0,22:6 PC or 18:0,18:1 PC SUVs for 0, 10 min, 1 hr and 2 hr and the extent of lipid incorporation quantified. A nearly linear increase in DHA levels (from 0 to 5.2%) occurred over this time period. These experiments indicated that as the level of DHA increased in the tumor cell, there was a concomitant increase in membrane permeability relative to 18:0,18:1 PC and a control group (no phospholipid). The relationship between incorporation of DHA and domains that may influence immunological epitopes was examined by MC540 emission. The presence of DHA in the tumor cell membrane (3.4%) increased MC540 liquid crystalline intensity at reduced temperatures (20°C) indicating that the presence of DHA may form domains within the tumor cell membrane. This hypothesis is substantiated

by alterations in the immunological epitopes of H-2^b; binding of the monoclonal Ab 3-83P (anti-K^k, D^k, K^b) to EL4 tumor cells (H-2^b) decreased with increasing DHA concentrations while binding of 28-8-6S (anti K^b, D^b) was increased with increasing DHA. Because DHA was the only lipid added to the membrane, and comprises only a small fraction (<10%) of the total fatty acids, alterations in these epitopes could likely be due to the congregation of DHA containing lipids around these proteins.

The final process examined, the effects of DHA on the aging process, was carried out by feeding young (1 month) and old (22 months) CBA mice a diet that consisted of either 10% menhaden oil (MO) or 10% hydrogenated coconut oil (HCO). Mice were fed the HCO diet for a period of 11 weeks, after which one-half of the mice were placed on the MO diet while the others remained on the HCO diet. After 3 weeks the livers were excised and mitochondria isolated and monitored for alterations due to diet in ATP content, respiratory control index (RCI), state 3 respiration rate, phosphate uptake and mean molecular area. Both young and old MO fed mice had substantial decreases in mitochondrial total ATP content, RCI, state 3 respiration rate and phosphate uptake relative to the HCO fed mice. Mean molecular area of mitochondrial lipids increased upon addition of DHA to the diets of young and old mice when compared to the HCO fed group. Because the presence of the omega-3 (21.7% DHA-young MO; 15.5% DHA-old MO) was severely affecting the bioenergetic process, it was important to understand if the results were influenced by the other fatty acids in the MO diet or were a result of DHA. We therefore monitored the RCI of young mouse liver mitochondria that were fused with either 18:0,22:6 PC or 18:0,18:1 PC. As the level of DHA increased in the membrane of the mitochondria there was a significant drop in the

RCI compared to the 18:1 fused group. It is therefore concluded that the presence of DHA in the diets of the mice did not reverse the deleterious effects of aging on mitochondria bioenergtics.