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Docosahexaenoic acid enhances 2-deoxyglucose treatment and antagonizes Metformin treatment in the reduction of intracellular ATP in breast cancer

A thesis submitted in partial fulfillment of the requirements for the degree of

BACHELOR OF SCIENCE, BIOCHEMISTRY AND MOLECULAR BIOLOGY

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May, 2015
We recommend that the thesis prepared under our supervision by

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entitled

Docosahexaenoic acid enhances 2-deoxyglucose treatment and antagonizes Metformin treatment in the reduction of intracellular ATP in breast cancer

be accepted in partial fulfillment of the requirements for the degree of

BACHELOR OF SCIENCE, BIOCHEMISTRY AND MOLECULAR BIOLOGY

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[May, 2015]
Abstract:

Epidemiological studies have shown that diets rich in omega-3 polyunsaturated fatty acids (PUFAs) correlate with lower incidence of cancer. Docosahexaenoic acid (DHA), an omega-3 PUFA was shown to suppress a wide range of cancer subtypes by inhibiting adenosine triphosphate (ATP), which thereby induces metabolic stress within the cell. By deregulating the cellular energetics of cancer cells, DHA has been shown to express tumor-suppressing effects. Other clinically relevant drugs that have proven effectiveness in targeting cancer metabolism include Metformin and 2-deoxyglucose (2DG). While both display their tumor-suppressing effects by reducing intracellular ATP, they do so by different mechanisms. Metformin targets the electron transport chain, while 2DG inhibits glycolysis. This study investigated the combinatorial effects of DHA with Metformin, as well as with 2DG. DHA was shown to enhance the effects of 2DG, but it displayed antagonistic behavior when treated in tandem with Metformin. Although DHA, Metformin, and 2DG each display similar results in ATP inhibition when treated alone, these results suggest that DHA’s ability to enhance the efficacy of other drugs is dependent on factors that may be related to pathway, mechanism, or localization.
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Introduction:

The effects of omega-3 polyunsaturated fatty acids (PUFAs) on health have been widely reported, and are generally shown to be beneficial. Their many remedial properties include the ability to improve cardiac function [1], modulate overactive immune responses [2], and aid in neurogenesis [3]. Omega-3 PUFAs have also been reported as possible therapeutic agents in cancer [4], with docosahexaenoic acid (DHA; C22:6 n-3) being shown as the most potent tumor suppressor [5]. Different mechanisms have been proposed as to how DHA is implementing its anti-cancerous effects, including bioenergetics metabolism [6], alterations of signaling by disruption of the lipid raft [7,8], and oxidative stress [9]. As more prominent research is being done to study DHA’s mechanism of action, a recent study in our laboratory has attributed much of its anti-cancerous effects to its ability to induce metabolic stress in cancer.

Cancer metabolism has increasingly been targeted due to the effectiveness of its treatment. Metformin, generally known for its effects on diabetes, has been shown to have inhibitory effects on cancer metabolism as well [10]. It decreases intracellular ATP levels by targeting complex I of the electron transport chain, which thereby increases AMP levels, leading to activation of AMPK [11]. This particular effect on cell metabolism illustrates metformin’s dual role in both diabetes treatment, as well as cancer treatment.

Another drug that targets cellular energetics is 2-deoxyglucose (2DG). Rather than targeting the mitochondria, 2DG inhibits glycolysis [12]. It acts by competitively inhibiting glucose-6-phosphate at the hexokinase step. Normally, glucose is
phosphorylated by hexokinase to form glucose-6-phosphate, but in the presence of 2DG, hexokinase is used to produce 2-deoxyglucose-6-phosphate, which is unable to continue through glycolysis [13]. Less ATP is generated with greater buildup of 2-deoxyglucose-6-phosphate due to the inability for glycolysis to run to completion. While 2DG has been shown to be an effective agent in treating cellular metabolism, high doses are toxic to the host. In order to offset these side effects, combination therapy has been explored to maintain the potency of 2DG while offsetting its side effects.

Studies in our laboratory has shown that DHA supplementation on the BT-474 human breast ductal carcinoma, MDA-MB-231 breast adenocarcinoma, and A549 human lung adenocarcinoma cell lines resulted in reduction of intracellular ATP, and activation of AMPK. Although downstream targets have yet to be studied, research has shown that DHA inhibits mTOR via AMPK activation [14].

Testing for synergism has been a popular test in recent years due to the clinical effectiveness of using lower dosage drugs in combination to further influence a target. Rather than test combinatorial effects of drugs, this study displays a unique characteristic by investigating the effects of clinically relevant drugs with a natural component of the human diet. The study investigates whether DHA can enhance the efficacy of Metformin and 2DG in the treatment of cancer by reducing intracellular ATP.
Materials and Methods:

Cell Lines and Reagents:

BT-474 mammary ductal carcinoma cells, MDA-MB-231 mammary adenocarcinoma cells, and A549 human lung adenocarcinoma cells were purchased from ATCC (Manassas, VA). Fatty acids, linoleic acid (LA; C18:2n-6) and docosahexaenoic acid (DHA; C22:6n-3) were purchased from Sigma Aldrich (St. Louis, MO). Metformin hydrochloride (Sigma Aldrich) dissolved in sterile H$_2$O. 2-deoxy-D-glucose (Sigma Aldrich) was dissolved in sterile H$_2$O.

Cell Culture:

Breast BT-474 cells were cultured in HybriCare culture medium (ATCC) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT). Breast MDA-MB-231 and lung A549 were cultured in RPMI-1640 (Thermo Scientific, Rochester, NY) supplemented with 10% FBS (Hyclone).

ATP Assay:

The CellTiter-Glo Luminescent assay (Promega, Madison, WI) kit was used per manufacture instructions. Cells were plated in white clear-bottom 96-well plates and after 72 hour culture period with respective treatments, had CellTiter-Glo Luminescent assay reagent added to it. Luminescence was read on a SpectraMax M5 plate reader (Molecular Devices LLC, Sunnyvale, CA).
Results:

**Total intracellular ATP in response to PUFA treatment**

Figure 1: Determining the effective concentration of DHA on ATP reduction. Breast BT-474, MDA-MB-231, and lung A549 cells were seeded for 24 hours prior to PUFA treatment, 2DG treatment, or an equal volume of EtOH control. After 48 hours of PUFA and/or 2DG treatment, the % ATP luminescence was determined by CellTiter-Glo® Luminescent assay (Promega, Madison, WI) and data represents percentage ATP luminescence relative to control. *All experiments were done in triplicate and data represents n=3 independent experiments.*
Figure 2: Determining the effective concentration of 2-deoxyglucose on ATP reduction. Breast BT-474, MDA-MB-231, and lung A549 cells were seeded with 2DG for 3, 6, 12, 24, and 48 hours. After a 72 hour culture period, the % ATP luminescence was determined by CellTiter-Glo® Luminescent assay (Promega, Madison, WI). All experiments represent at least n=3. Abbreviations: 2DG (2-deoxyglucose).
Figure 3: Determining the optimal time treatment and effective concentration of Metformin on ATP reduction. [A] Breast BT-474 and [B] MDA-MB-231 cells were seeded with 1.25, 2.5, and 5 mM Metformin for 12, 24, and 48 hours. After a 72 hour culture period, the % ATP luminescence was determined by CellTiter-Glo® Luminescent assay (Promega, Madison, WI).
Figure 4: Determining the optimal time treatment and effective concentration of Metformin on ATP reduction. Breast BT-474, MDA-MB-231, and lung A549 cells were seeded for 24 hours prior to PUFA treatment, 2DG treatment, or an equal volume of EtOH control. After 48 hours of PUFA and/or 2DG treatment, the % ATP luminescence was determined by CellTiter-Glo® Luminescent assay (Promega, Madison, WI) and data represents percentage ATP luminescence relative to control. All Experiments were done in triplicate and data represents n=3 independent experiments.
Figure 5: Determining the effects of DHA and Metformin on ATP reduction. Breast BT-474 cells were seeded for 24 hours prior to PUFA treatment, and Metformin treatment. After 48 hours of PUFA and/or Metformin treatment, the % ATP luminescence was determined by CellTiter-Glo® Luminescent assay (Promega, Madison, WI) and data represents percentage ATP luminescence relative to control.
Discussion:

While many studies continue to investigate the mechanism for how DHA inhibits tumor growth, our results display DHA’s ability to deregulate cellular energetics by inhibiting ATP in both breast and lung cancer [Figure 1]. Its effects are similar in potency when compared to 2DG [Figure 2], and Metformin [Figure 3]. Although each treatment shows very similar effects on ATP reduction of cancer cells, their effects are inconsistent when treated in tandem. DHA was shown to enhance the efficacy of 2DG [Figure 4], but it surprisingly antagonized the effects of Metformin [Figure 5]. In order to determine the reason for such inconsistencies between these combinations, an in depth analysis of each drugs mechanism was investigated. 2DG’s ATP reducing effects are due to the inhibition glycolysis. Metformin’s effects are localized in complex 1 of the electron transport chain in the mitochondria. Interestingly, 2DG and Metformin have been shown to inhibit tumor growth synergistically [15]. Perhaps the synergistic relationship is due to the targeting of two different pathways (glycolysis and oxidative phosphorylation) that both lead to ATP synthesis. Other studies have displayed very similar effects from DHA and Metformin. Both inhibit ATP, induce AMPK, and decrease levels of mTOR. Due to such similarities, the potential for competition exists when both treatments occur in combination. Perhaps DHA’s effects are localized in the mitochondria as well, and it competitively inhibits the effects Metformin. Further studies will be done to determine DHA’s effects on 2DG and Metformin on downstream targets, including: AMPK, mTOR, and 4EBP1.
References:


