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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

Effect of ketone bodies on viability of human breast cancer cells (MCF-7)

Keton cisimlerinin insan meme kanseri hücrelerinde (MCF-7) canlılık üzerine etkileri

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ABSTRACT

Objective: Cancer cells exhibit an elevated glycolytic phenotype under aerobic conditions, which is known as the Warburg effect. Recent studies have also shown that cancer cells are glucose-dependent and cannot use ketone bodies as a primary source of energy. In this study, we have investigated the effects of ketone bodies on viability of breast cancer cells considering that breast cancer cells would not use ketone bodies as a primary energy source.

Materials and Methods: In this study we have used MCF-7 cells, which are breast cancer cells that cannot use ketone bodies as a primary energy source and human foreskin fibroblast cells (HFF) as controls. We measured cell viability in both cells cultured in the presence or absence of glucose as well as the ketone bodies acetoacetate and beta-hydroxybutyrate.

Results: Cell viability was significantly decreased in response to ketone bodies compared with control media in MCF-7 cells whereas in control cells (HFF) cell viability was not changed.

Conclusion: In light of the data obtained, we suggest that dietary manipulation with the use of ketone bodies may be a new therapeutic strategy for breast cancer.

Keywords: Acetoacetate, Beta-hydroxybutyrate, ketone bodies, MCF-7 cancer cells

ÖZ

Amaç: Kanser hücreleri aerobik koşullar altında Warburg etkisi olarak bilinen artmış glikolitik fenotipini sergilerler. Ayrıca, son zamanlarda yapılan çalışmalar, kanser hücrelerinin glikoza bağımlı olduğunu ve keton cisimlerini birincil bir enerji kaynağı olarak kullanamadığını göstermiştir. Bu çalışmada, insan meme kanseri hücrelerinin birincil enerji kaynağı olarak keton cisimlerini kullanamayacağını varsaydık ve keton cisimlerinin hücre canlılığı üzerine etkisini araştırdık.

Gereç ve Yöntem: Bu çalışmada insan meme kanseri hücre hattı (MCF-7) ve insan foreskin fibroblast hücre hattı (HFF) kullanıldı. Her iki hücre hattında glukoz içeren ve içermeyen ortamlarda keton cisimleri olan asetoasetat ve beta-hidroksibütiratın hücre canlılığına etkisini inceledik.

Bulgular: Keton cisimlerini içeren ortamda MCF-7 hücrelerinde hücre canlılığı kontrol ortamı ile karşılaştırıldığında azalırken, kontrol olarak kullanılan HFF hücrelerinde herhangi bir değişiklik görülmedi.

Sonuç: Elde edilen veriler ışığında, keton cisimlerinin kullanılması ile gerçekleştirilecek beslenmenin meme kanseri tedavisinde yeni bir strateji olabileceğini düşünmekteyiz.

Anahtar kelimeler: Asetoasetat, Beta-hidroksibütirat, Keton cisimleri, MCF-7 kanser hücreleri

Introduction

Ketone bodies are effective energy substrates for healthy extrahepatic tissues. However, cancer cells cannot use them efficiently for energy [1]. Mitochondrial pathology observed in cancer cells include decreased number of mitochondria, swelling, mutations in mtDNA, altered membrane potential and abnormal enzyme presence or function [2-4]. All of these defects in mitochondrial structure and function weaken respiratory capacity and make it difficult to rely on substrate-level phosphorylation for survival [5]. Ketone bodies are metabolized only in mitochondria, thus cancer

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cells with mitochondrial dysfunction cannot efficiently metabolize them for energy [6].

Ketone metabolism in healthy whole cells may inhibit cancer cell growth by creating a less favorable redox environment for survival. Ketone bodies have properties that can disrupt the survival and proliferation of cancer cells [7]. Pyruvate formed by glycolysis is directed to the mitochondria for tricarboxylic acid (TCA) cycle and oxidative metabolism in normal cells. However, in cancer cells pyruvate is often converted to lactic acid and then used in the fermentation pathway [8]. This provides a number of advantages to cancer cells. One of these is the bypassing of mitochondrial oxidative metabolism and concomitant reactive oxygen species (ROS) formation. Since cancer cells show a higher level of oxidation than normal cells, this is an advantage in survival making them less susceptible to ROS-mediated apoptotic stimuli [9]. Ketone bodies block the main pathway of energy production for cancer cells by inhibiting glycolysis [10]. Cancer cells are highly susceptible to minor changes in redox status with success in increased ROS environment [11]. Ketones also lower mitochondrial ROS production and develop endogenous antioxidant defenses in normal cells, but not in cancer cells [12].

In this study, we have investigated the effects of ketone bodies on viability of breast cancer cells, considering that breast cancer cells would not use ketone bodies as a primary energy source. We measured cell viability in breast cancer cells cultured in the presence and absence of glucose, and ketone bodies acetoacetate (AA) and beta-hydroxybutyrate (BHB).

Materials and Methods

Stock solutions of AA and BHB were dissolved in 10 mM phosphate buffer saline as described by Noh et al [13]. Human breast cancer cells (MCF-7) and human foreskin fibroblast cells (HFF) were obtained from American Type Culture Collection (ATCC, USA) and were maintained under standard culture conditions. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Sigma, USA) containing 1% penicillin and streptomycin.

Different groups and conditions used in the experiments

Control group 1: DMEM with high glucose (4.5 g/L); Control group 2: DMEM with low glucose (1.125 g/L) Control group 3: DMEM without glucose (glucose-free); Ketone body groups (AA or BHB): glucose-free media supplemented with either 10 and 20 mM AA or BHB.

Measurement of cell viability

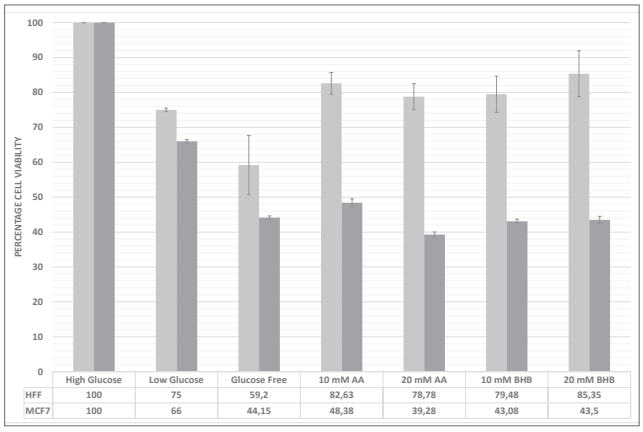
The cell cycle for the MCF-7 cells is on the average 21 hours [14]. Thus, we chose 24 hours for measurement of cell viability with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) which is a stable tetrazolium salt reduced in living cells with NAD(P)H. The amount of formazan crystals formed is directly proportional to the number of living cells. HFF and MCF-7 cells were plated on 24 well plates containing 1 ml of medium (50,000 cells/well). Cells were incubated with either 10 and 20 mM AA or 10 and 20 mM BHB at 37°C. MTT solution was added 24 hours after the ketone body application. After further incubation for 2 hours at 37°C absorbances at 590 nm was measured using a microplate reader. Percentage cell viability (%) of each group was calculated.

Statistics

All experiments were performed in triplicate and the results are shown as mean ± standard deviation. ANOVA test was done using GraphPadPrism software and $p < 0.05$ was taken as the significance.

Results

In this study, we determined whether cells could use ketone bodies as an alternative to glucose by culturing the cells in ketogenic media and measuring the cell viability. We have used AA and BHB as ketone bodies. As shown in Figure 1, we found significant differences in cell viability of HFF and MCF-7 cells under different culture conditions. HFF cells showed a small decrease in viability when cultured in glucose-free media. Addition of AA or BHB also resulted in a small increase in viability, but the difference was not statistically significant. In contrast, when MCF-7 cells were subjected to glucose-free media, their viability decreased significantly compared to controls. Additionally, this effect was not reversed by ketone bodies AA and BHB.



	High Glucose	Low Glucose	Glucose Free	10 mM AA	20 mM AA	10 mM BHB	20 mM BHB
HFF	100	75	59,2	82,63	78,78	79,48	85,35
MCF7	100	66	44,15	48,38	39,28	43,08	43,5

Figure 1. Percentage cell viability values for HFF and MCF-7 cells 24 hours after incubation at different experimental conditions.

High glucose: DMEM containing 4.5 g/L glucose; Low glucose: DMEM containing 1.125 g/L glucose; Glucose free: DMEM without glucose; AA and BHB: either 10 or 20 mM in DMEM without glucose

Values represent mean ± SD.

Discussion

Breast cancer cells (MCF-7 cells) subjected to glucose-free media had a significant decrease in viability that could not be reversed by the addition of ketone bodies. This means that cells were not rescued by the addition of the AA or BHB. Thus, our data support the hypothesis that, like other cancers of breast origin, MCF-7 cells lack the ability to use ketone bodies as an energy source.

Succinyl-coenzyme A transferase is an enzyme which converts ketone bodies to acetyl-CoA and catalyzes the rate-limiting step in the metabolic processing of ketone bodies for energy production. Skinner and colleagues observed that viability of the human neuroblastoma cells (SK-N-AS cells) decreased with ketone body treatment [1]. They also reported lower expression of the above mentioned enzyme in SK-N-AS cells compared to HFF cells. Poff and colleagues observed inhibition of ATP production by AA and proliferation in seven aggressive human colon and breast cancer cell lines, but did no effect on proliferation in healthy primary fibroblasts [7]. Similarly, BHB inhibited the proliferation of transformed lymphoblast, HeLa cells and melanoma cells in a dose-dependent manner up to 20 mM. Additionally, BHB and AA were shown to reduce viability in neuroblastoma cells, but had no effect on fibroblasts. In contrast, Martinez-Outschoorn and colleagues observed an increase in viability of MCF-7 cells with ketone body treatment in their co-culture study with MCF-7 and fibroblast cell lines [15]. They also observed that the fibroblast cell lines formed acetyl-CoA using ketone bodies and the MCF-7 cell lines maintained their viability using the acetyl-CoA produced by the fibroblast cell lines.

In conclusion, our results show a significant reduction in cell viability of MCF-7 breast cancer cells supporting the Warburg effect. However, the molecular mechanism of the changes needs to be further identified. We also believe that use of a ketogenic diet might create new therapeutic approaches in the treatment of different forms of cancer.

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