

Inhibitory effect of carbonic anhydrase inhibitors on the de novo lipogenesis. A study with ¹³C-NMR spectroscopy

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Introduction

Obesity is a widespread disease in both the developed and developing countries, which affects over 300 million persons. There are some pharmacological approaches for treatment of obesity at this time. A new therapeutic approach for treatment and/or prophylaxis of obesity, which can complement the previously known form of therapy, intends the inhibition of de novo lipogenesis (DNL) from carbohydrate in the mammalian organism and is based on the inhibition of carbonic anhydrases (CAs) isozymes and involved in several steps of de novo lipogenesis, localized in the mitochondria and the cytosol of cells. These enzymes catalyze a very simple physiological reaction, the interconversion of carbon dioxide and bicarbonate. It is comprehensible that CA V and CA II subsequently affect pyruvate carboxylation. Sulphonamide/ Sulphamate are carbonic anhydrase inhibitors (CAIs) and good candidate to inhibit DNL on the step of pyruvate carboxylation in mitochondria. Inhibition of CA will reduce the influx into the anaplerotic activity of pyruvate carboxylase (pc) and subsequently into the citric acid (TCA) cycle [1], which will modify the intermediates of the TCA cycle and its effluxes (in particular glutamate) and lipid synthesis [2]. It has been observed that patients treated with the antiepileptic drug Topiramate, which exhibits also CA inhibitory effects, loose weight suggesting an interference with the energy metabolism. In view of the important role of pyruvate carboxylase in DNL, we examined the effect of CAIs i.e., topiramate (TPM), ethoxzolamide (ETZ) and acetazolamide (ACT) on bicarbonate fixation and the anaplerotic reaction in 3T3-L1 cells by means of ¹³C-NMR spectroscopy. Figure 1 show a schematic representation in which way CAIs might affect metabolites that will be used for de novo lipid synthesis and glutamate synthesis in 3T3-L1 cells [3].

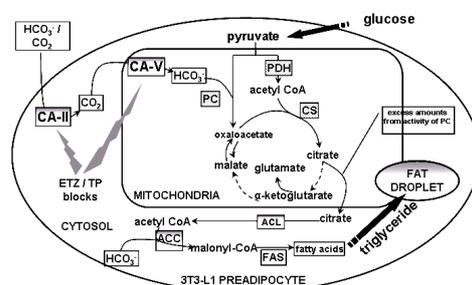


Figure 1. Supposed role of carbonic anhydrase activity and CAIs mechanism in de novo lipid synthesis in adipocytes.

Materials and methods

The 3T3-L1 cells were grown to confluence in 5-cm culture dishes and cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum and 1% penicillin/streptomycin and kept in an incubator with a humidified atmosphere of 10% CO₂ in air at 37° C. The cells were incubated for 24h with serum-free DMEM culture medium containing 4 mM glutamine and 5 mM [U-¹³C]glucose or [2-¹³C]acetate as tracer, in the presence or absence of various CAIs. i.e. TPM, (100 μM), ACT (150 μM), and ETZ (13μM). After 24h incubation, the cells were immediately washed twice with 5 ml ice-cold saline, frozen in liquid nitrogen and extracted with ice-cold 0.9 M perchloric acid. The suspension was centrifuged and the supernatant was collected, neutralized with KOH, and lyophilized (cell extracts). The pellet, containing lipids, was redissolved in 5 ml water and neutralized with KOH and lyophilized. The cell- and lipid-extracts were redissolved in 0.6 ml D₂O and 1.0 ml CD₃Cl/CD₃OD (2:1) respectively for NMR analysis. ¹H- and ¹³C-NMR spectra were recorded on Bruker DRX-600.

Results

The visible products of pyruvate carboxylation via anaplerotic, in the cell extract are either [2,3,4,5-¹³C] and [U-¹³C]glutamate (in relation to the oxidative formation of [4,5-¹³C]glutamate) and double or multiple labelled fatty acid residues. ¹³C-NMR analysis of the multiplet of C-4 glutamate and doublet at F₀ of lipid show a ca inhibition induced decrease the anaplerotic activity of pc activity causing lower concentrations of [2,3,4,5-¹³C] and [U-¹³C]glutamate to and reduced lipid synthesis. These values are shown in table 1.

Table 1. % ¹³C enrichment versus control. Values are means± SD (n=3-4)

% of control	TPM, 100μM, 24h	ACT, 150 μM, 24h	ETZ, 13μM, 24h
[2,3,4,5- ¹³ C]-, [U- ¹³ C]glu via pc	70.3±11.7	58.7±7.8	57.5±4.9
De novo lipogenesis	76.6±4.8	67.1±16	73.8±11

We have also used [2-¹³C]acetate as tracer to study the effect of CAIs on related fatty acid synthetase enzymes in cytosol. [2-¹³C]acetate will be converted into [2-¹³C]acetyl-CoA in cytosol. Thus the cells can use these units to fatty acid synthesis directly, which the acetyl-CoA units from glucose will be formed in mitochondria and enters the TCA cycle forming citrate. The citrate is transported into the cytosol to serve as a substrate for acetyl-CoA and DNL. The results from this study show that CAIs have no effect on de novo lipid synthesis from [2-¹³C]acetate.

Discussion

The CAIs caused decreased pyruvate-carboxylase-mediated carbon fixation and de novo lipogenesis in 3T3-L1 cells. As shown in table 1 the quantitative reduction of TCA cycle anaplerosis and lipid biosynthesis are different suggesting that the effects on the intramitochondrial (CA V) and cytosolic (CA II) isozymes are different. It is hypothesized that CAIs inhibit pyruvate carboxylation in 3T3-L1 cells by limiting the supply of bicarbonate to this enzyme. If the effects of CAIs were on acetyl-CoA carboxylase, an decrease in lipid synthesis from [2-¹³C]acetate in the presence of CAIs would be expected, which we didn't see. Consequently the decrease of lipid synthesis can be related to the decrease of CA activity, which explains the weight loss of patients treat with the antiepileptic drug, topiramate.

References:

[1]. Hazen et al. CJ. Dev Neurosci. 1997;19:162-171 [2]. Lynch et. al.. Biochem. J. 1995;310:197-202. [3] Stacy A. Hazen, et al. FASEB, 10, 481- 490, (1996).