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RESEARCH ARTICLE

CONCENTRATION-DEPENDENT INHIBITORY EFFECT OF CALCIUM FRUCTOBORATE ON SIRT1 ENZYME ACTIVITY: A COMPARATIVE ANALYSIS WITH BORIC ACID

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ABSTRACT

Purpose: SIRT1 functions in regulatory mechanisms including cellular aging, apoptosis, glucose and lipid metabolism, oxidative stress and inflammation. Studies show that SIRT1 inhibitors may affect various types of cancer and metabolic disorders, making SIRT1 an important therapeutic target. Boric acid is an inorganic boron compound with antimicrobial, antifungal and potential anticancer effects. Calcium fructoborate, another boron compound found in vegetables and fruits, has anti-inflammatory, antioxidant and anti-tumor effects. This study aimed to investigate the inhibitory potential of calcium fructoborate on SIRT1 activity and to evaluate its possible use as a chemical tool for future studies targeting SIRT1 regulation.
Material and Methods: In this study, the effect of calcium fructoborate on SIRT1 activity was investigated by a fluorescence-based method using human recombinant SIRT1.
Results: Calcium fructoborate inhibited SIRT1 activity in a concentration-dependent manner. Calcium fructoborate significantly inhibited SIRT1 activity by 42% at a concentration of 20 mM ($p < 0.001$). Boric acid did not produce a statistically significant or consistent concentration-dependent change in SIRT1 activity within the tested concentration range.
Conclusion: These preliminary cell-free findings suggest that calcium fructoborate inhibits SIRT1 activity, indicating its potential as a chemical tool for studying the biological functions of SIRT1. However, the biological relevance of this effect and its potential therapeutic implications require confirmation through further studies in cellular and in vivo models.

Keywords: calcium fructoborate, boric acid, human recombinant SIRT1, enzyme activity

INTRODUCTION

Sirtuins are a highly evolutionarily conserved family of enzymes, so named because of their structural similarity to the silent information regulator 2 (*Sir2*) gene identified in yeast (*Saccharomyces cerevisiae*). These enzymes are nicotinamide adenine dinucleotide (NAD+) dependent proteins that exert deacetylase activity on acetylated lysyl residues. The enzymatic activity of sirtuins is sensitive to intracellular levels of NAD+ and its reduced form, NADH. Thus, the functionality of these enzymes is closely linked to cellular metabolic status, and they play important roles in energy balance, aging, inflammation and various disease processes (1). Histone deacetylase Sirtuin 1 (SIRT1) plays essential roles in various processes. SIRT1 regulates multiple

cellular processes by deacetylating numerous proteins and genes, including those involved in cell survival, differentiation, metabolism, DNA repair, inflammation, neuroprotection, and longevity (2). Boron, a naturally occurring element, is involved in various biological processes including mineral metabolism, maintenance of cell membrane integrity, hormone receptor function, and transmembrane signaling pathways (3).

While boron is not classified as an essential nutrient for human nutrition, it is increasingly recognized as a bioactive dietary component that contributes significantly to overall health. Growing evidence suggests that physiological levels of boron intake elicit various biochemical and functional responses, supporting bone development and maintenance, enhancing cognitive function, and potentially lowering the risk of certain cancers (4). According to the World Health Organization (WHO), daily boron consumption typically includes approximately 1.2 mg from dietary sources and an additional 0.2–0.6 mg from drinking water (5).

Boric acid is an inorganic boron compound that occurs naturally and exhibits weakly acidic properties; it has been used in various biological and pharmacological applications due to its antimicrobial, antifungal, antiseptic, and potential anticancer effects (6). CaFB is an organic boron-containing sugar-borate complex that naturally occurs in various plant-based foods, including vegetables like celery and broccoli, and fruits such as grapes and plums. Among boron compounds, sugar-borate esters are considered to be the most bioavailable form for efficient cellular absorption (7). The enzymatic activity of SIRT1 refers to its ability to exert functional effects by deacetylating its substrates. The measurement of SIRT1 activity is critical for assessing the functional status of this enzyme, particularly in pathophysiological conditions such as metabolic disorders, ageing, obesity, and cancer. Enzyme activity assays provide a fundamental biochemical approach to evaluating the efficacy of pharmacological agents targeting SIRT1 and to developing therapeutic strategies based on NAD+ metabolism (8). Regarding cancer and metabolic disorders, several studies have suggested that inhibiting SIRT1 and other sirtuin isoforms could provide therapeutic benefits (9, 10). However, the direct relation between boron-containing compounds such as calcium fructoborate and SIRT1 enzyme activity has not yet been elucidated. Given SIRT1's role in redox regulation and inflammatory processes, and the reported antioxidant and anti-inflammatory properties of boron compounds, a potential functional interaction between these systems may be suggested. We hypothesized that calcium fructoborate may modulate SIRT1 activity due to its reported biological effects. In this study, we aimed to investigate the potential inhibitory or activating effects of BA and CaFB on SIRT1 enzyme activity using a fluorometric method.

MATERIALS AND METHODS

Calcium Fructoborate (CaFB)

The CaFB compound used in this study was synthesized by Dr. Mehmet Ali Kısacam following the patented method described by Miljkovic (11). Acetone, boric acid, calcium carbonate (CaCO3), and fructose obtained from Sigma Chemicals were used in the synthesis procedure. The characterization of CaFB was previously performed and reported by Kısaçam et al. (12) using Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC), Nuclear Magnetic Resonance (NMR), and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Analytical characterization confirmed that the synthesized product had the expected chemical structure with a purity of 99.9%. For experimental applications, CaFB stock solutions were prepared in assay buffer at a final concentration of 100 mM and sterilized using a 0.22 µm membrane filter (Biosharp). Working solutions at concentrations of 2, 5, 10, and 20 mM were then prepared through serial dilution of the primary stock.

Boric Acid (BA)

Boric acid (BA) was obtained from Merck (CAS No: 10043-35-3, Germany). Stock solutions of BA were prepared in assay buffer at a concentration of 10 mg/mL and sterilized by filtration. From this stock solution, serial dilutions were performed to obtain working concentrations of 100, 250, 500, and 1000 µg/mL in assay buffer, corresponding to approximately 1.6, 4.0, 8.1, and 16.2 mM, respectively (molar mass of boric acid = 61.83 g/mol). The resulting molar concentrations were within a range similar to that used for CaFB (2–20 mM). It also falls within a similar molar concentration range to those previously tested for CaFB and BA (0.45, 2.25, 5, 11.25, and 22.5 mM) in MDA-MB-231 breast cancer cells in vitro (6). However, a direct comparison on a boron-equivalent basis would require

consideration of the number of boron atoms per molecule of each compound.

SIRT1 Activity Assay

The SIRT1 Direct Fluorescence Screening Assay (10010401, Cayman Chemical, USA) is a fluorescence-based method used to screen SIRT1 inhibitors or activators. The assay is carried out in two steps within the same microplate. In the first step, a substrate containing the p53 sequence is incubated with human recombinant SIRT1 in the presence of the co-substrate NAD+, leading to deacetylation of the substrate. In the second step, the treated substrate is exposed to a developer solution, which enables the release of a fluorescent product. Fluorescence was subsequently recorded at excitation wavelengths of 350–360 nm and emission wavelengths of 450–465 nm. This biochemical approach enabled precise measurement of the direct inhibitory effect of CaFB on the deacetylation of a p53-based substrate, independent of cellular metabolic interference.

CaFB and BA compounds were prepared by dissolving them in the assay buffer. CaFB at concentrations of 2 mM, 5 mM, 10 mM, 20 mM, and BA at concentrations of 100 µg/mL, 250 µg/mL, 500 µg/mL, 1000 µg/mL were added into the 50 µL working volume. Assay buffer, recombinant SIRT1, and respective compound were added into relevant wells, and the assay was tested in technical triplicate across three independent experiments. Compound-only fluorescence controls for CaFB and BA were not performed. The reaction was then initiated by adding 15 µL of substrate solution to all wells. The plate was covered and incubated on the shaker for 45 min at room temperature. 50 µL of the stop solution was added and the mixture was incubated again for 30 min. Fluorescence reading was performed at 360/460 nm. To calculate the % inhibition/activation for each sample, the sample value was subtracted from the 100% initial activity value, and the result was expressed as a ratio to the 100% initial activity value.

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism 9 (GraphPad Software, Inc., San Diego, CA, USA) and IBM SPSS Statistics version 29.0 (SPSS Inc., Chicago, IL, USA). To evaluate differences among multiple groups, a one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test for multiple comparisons was employed. All experimental data are derived from three independent experiments, each performed with three technical replicates per condition, and are expressed as the mean ± standard deviation (SD). A p-value of less than 0.05 was established as the threshold for statistical significance.

RESULTS

Effect of Boron Treatment on SIRT1 Activity

A fluorescence-based method was used to determine SIRT1 activity. According to these findings, the CaFB compound inhibited SIRT1 activity in a concentration-dependent manner. SIRT1 activity was statistically significantly inhibited by 17% ($p < 0.01$) in the C1 group, 24% ($p < 0.01$) in the C2 group, 31% ($p < 0.001$) in the C3 group, and 42% ($p < 0.001$) in the C4 group (Figure 1). In the group treated with boric acid (BA), a numerically lower mean SIRT1 activity was observed at some concentrations; however, BA did not produce a statistically significant or consistent concentration-dependent change in SIRT1 activity within the tested range (Figure 2).

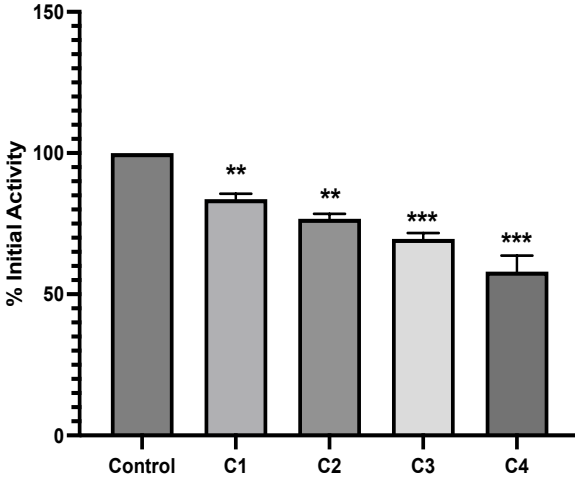


Figure 1. SIRT1 activity following calcium fructoborate (CaFB) treatment. CaFB was tested at 2, 5, 10, and 20 mM (C1–C4). Data are presented as mean ± standard deviation (SD). Statistical significance was assessed using one-way ANOVA followed by Dunnett's post-hoc test. **$p < 0.01$; ***$p < 0.001$ vs. control.

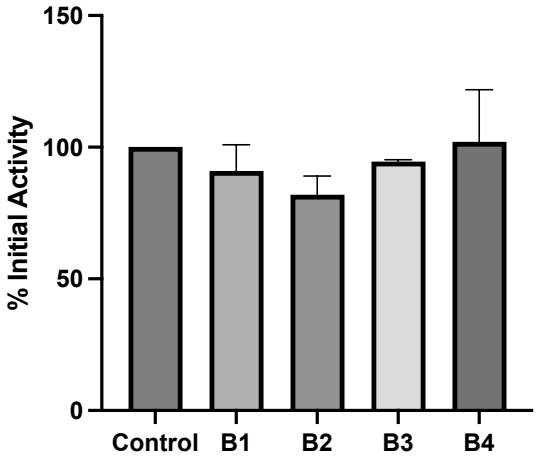


Figure 2. SIRT1 activity following boric acid (BA) treatment. BA was tested at 100, 250, 500, and 1000 µg/mL (B1–B4). Data are presented as mean ± standard deviation (SD). Statistical significance was assessed using one-way ANOVA followed by Dunnett's post-hoc test.

DISCUSSION

In this study, CaFB and BA were evaluated as candidate modulators of SIRT1 activity. To our knowledge, this is the first study to examine the effect of CaFB on SIRT1 enzyme activity. BA showed no significant effect on SIRT1 activity, which may reflect a true lack of activity or an insufficient dose or exposure duration. CaFB, by contrast, may act as a more effective modulator: its natural sugar-borate ester structure could increase its protein-interaction capacity, consistent with reports that organic boron esters show higher membrane permeability and protein-binding affinity than inorganic boron forms (13). This structural property may have contributed to the concentration-dependent SIRT1 inhibition observed with CaFB; however, this interpretation remains hypothetical and requires confirmation through biophysical or structural studies.

Recent research shows that inhibition of SIRT1 by certain molecules can disrupt cancer cell survival pathways, making these agents potential candidates for combinatorial therapeutic approaches in oncology (14). In this context, SIRT1 inhibitors such as Tenovin-6 and EX-527 have been reported to induce apoptosis and modulate cancer-related pathways in different cancer cell models. Tenovin-6 has been shown to activate p53 and promote apoptosis in acute lymphoblastic leukemia cells, while EX-527 has been reported to enhance the response of pancreatic cancer cells to chemotherapy and to induce p53-mediated apoptosis in glioma models (15–17). These findings suggest that SIRT1 inhibition with CaFB may have relevant cellular consequences. However, it is not yet known whether CaFB produces similar effects, which will require further cellular studies.

The potential relevance of SIRT1 inhibition to p53-related cellular responses is also of interest. Both Tenovin-6 and EX-527 have been reported to affect p53-related responses in cancer cell models (15–17). In our assay, CaFB inhibited recombinant human SIRT1 activity in a concentration-dependent manner in a cell-free assay using a p53-derived peptide substrate. Although this finding demonstrates inhibition of SIRT1 activity under the tested conditions, it does not establish an effect of CaFB on p53 deacetylation or p53-dependent cellular pathways. Further molecular and cellular studies are therefore needed to determine whether the observed SIRT1 inhibition translates into changes in p53-related responses or other SIRT1-regulated cellular processes.

SIRT1 inhibition has also been investigated in metabolic disease models. In a diet-induced fatty liver model, EX-527 was reported to attenuate high-fat diet-induced hepatic steatosis and fibrosis in Zucker rats, with effects associated with the SIRT4/SMAD4-related regulatory axis (18). Although these findings cannot be directly extrapolated to CaFB, they support further investigation of whether CaFB-mediated SIRT1 inhibition may have biological relevance in metabolic conditions.

Pirouz et al. reported that CaFB can selectively accumulate in tumor tissue in a breast cancer model, suggesting its potential as a boron-carrying agent for boron neutron capture therapy (BNCT) (19). Together with the SIRT1 inhibition observed in the present cell-free study, these findings provide a basis for further investigation of the potential biological effects of CaFB in tumor models. However, whether CaFB-mediated SIRT1 inhibition occurs in cells or contributes to tumor responses, including in the context of BNCT, remains to be determined.

Limitations

This study has several limitations. First, the assay used recombinant human SIRT1 in a cell-free system, and neither the binding site nor the molecular mechanism of the observed inhibition was investigated. Second, potential fluorescence interference from CaFB and BA was not assessed using compound-only controls and cannot be

excluded. Third, the CaFB concentrations required for significant inhibition (2–20 mM) exceeded normal human blood boron levels (2–61 μM) (20) by three to four orders of magnitude, and whether such concentrations are physiologically or pharmacologically achievable in vivo remains unknown. Further cellular and in vivo studies are needed to address these limitations.

CONCLUSION

To the best of our knowledge, the present study is the first to demonstrate a concentration-dependent inhibitory effect of CaFB on recombinant human SIRT1 activity, providing preliminary biochemical evidence of an effect of a boron-based compound on SIRT1 activity. These findings suggest that CaFB may have potential as a candidate chemical tool for studying the biological functions of SIRT1. However, as the present findings are based on a cell-free experimental model, the biological relevance of the observed inhibition and its potential therapeutic implications have not yet been established. Further cellular and in vivo studies are required to validate these findings and to clarify the effects of CaFB on SIRT1-related biological processes and its potential therapeutic relevance.

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